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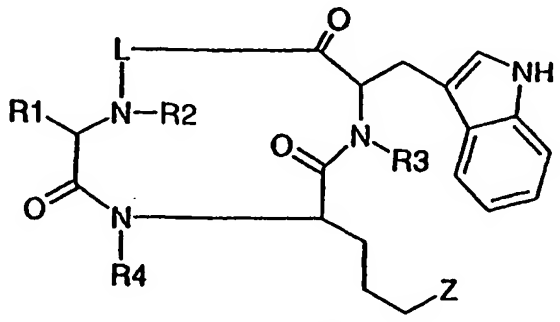
(54) Title: COMPOUNDS FOR CONTROL OF EATING, GROWTH AND BODY WEIGHT

(57) Abstract

The invention relates to compounds of general formula (1) or general formula (2) and their uses for the treatment of drug abuse, for the control of eating behaviour, body weight and growth and metabolism of animals, including humans.



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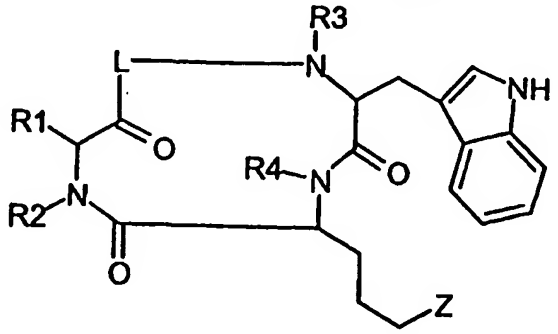

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COMPOUNDS FOR CONTROL OF EATING, GROWTH AND BODY WEIGHT

The present invention relates to new compounds which may be used for the control of eating behaviour, body weight and growth of animals, including humans. In particular the invention provides compounds for these applications which may be active upon administration in the periphery (e.g. intramuscularly, subcutaneously, intravenously, intraperitoneally, orally, topically, etc.). It is a further objective of the invention to provide compounds which may exert their effect on eating, body weight and/or growth by causing central effects on the brain. A further aspect of the invention is to provide compounds that may penetrate through the blood brain barrier allowing the administration of the compounds of the invention to the periphery, and still being capable of inducing effects within the central nervous system.

The invention also relates to compounds which may bind with high affinity to melanocyte stimulating hormone receptors.

The invention also relates to the methods for manufacture and pharmaceutical preparations of the compounds of the invention, as well as to their use for various medical and veterinary practices related to melanocyte stimulating hormone receptors.

Eating behaviour is regulated by a complex network of physiological regulatory pathways that involve both the central nervous system and peripheral sites. Peripherally released leptin and insulin are regarded as key mediators that act on hypothalamic sites. Moreover, within the central nervous system various regulatory factors are

involved, among which may be mentioned NPY (neuropeptide Y), orexins, CRF (Corticotropin-Releasing Factor) and melanocortic peptides (Schwartz; Nature Medicine 1998, 4, 385-386). These systems control the amount of food intake both in short and long term which may secondarily affect body weight, body fat mass and growth rate. Thus, e.g. NPY, administered intracerebroventricularly (icv) or directly into specific regions of the hypothalamus, is shown to dramatically increase food intake, rate of body weight increase as well as gain of total body fat (Stanley et al., Peptides 1986, 7, 1189-1192). Another system that is known to be involved in the control of eating and body weight homeostasis is the melanocortic system. Thus, injections of the melanocortic peptides α-MSH and ACTH(1-24), either icv or directly into the hypothalamus, was shown to markedly inhibit feeding (Poggioli et al., Peptides, 1986, 7, 843-848; Vergoni et al., Neuropeptides, 1986, 7, 153-158). The melanocortic peptides (melanocortins) are natural peptide hormones of animals and humans which are known to bind to MSH-receptors, which are termed MC-receptors. Examples of melanocortins, besides the α-MSH and ACTH, are β-MSH, γ-MSH, ACTH and peptide fragments of these.

A great leap forward in the understanding of the molecular basis for the action of melanocortins was taken a few years ago by the molecular cloning of genes encoding five different subtypes of MC-receptors termed MC1, MC2, MC3, MC4 and MC5 (Chhajlani and Wikberg 1992, FEBS Lett. 309, 417-420; Chhajlani et al., Biochem. Biophys. Res. Commun. 1993, 195, 866-873; Mountjoy et al., Science 1992, 257, 1248-1251; Gantz et al., J. Biol. Chem. 1993, 268, 8246-8250; Gantz et al., J. Biol. Chem. 1993, 268, 15174-15179; Griffon et al., Biophys. Res. Commun. 1994, 200, 1007-1014; WO 93/21316; WO 94/04674; US 5,622,860).

The MC-receptors belong to the class of G-protein coupled receptors which are all built from a single peptide chain forming 7 transmembrane domains. The five MC-receptors couple in a stimulatory fashion to cAMP. Of these the MC2-receptor is the ACTH-receptor whereas the others constitute subtypes of melanocyte stimulating hormone receptors (MSH-receptors). The various MC-receptors show distinct distributions in the body. For example high expression of MC2-receptors is present in the adrenal cortex (Xia et al., Cell Tissue Res. 1996, 286, 63-68), whereas the MC3 and MC4-receptors show distinct distributions in the brain including the hypothalamus (Low et al., Curr. Opin. Endocrinol. Diabetes. 1994, 1, 79-88). By contrast to the MC4-receptor which appears to be quite uniquely distributed only to the central nervous system (Low et al., Curr. Opin. Endocrinol. Diabetes. 1994, 1, 79-88), the MC3-receptor is also located to peripheral sites (Gantz et al., J. Biol. Chem. 1993, 268, 8246-8250). The MC1-receptor is present on melanocytes and melanoma cells (Low et al., Curr. Opin. Endocrinol. Diabetes. 1994, 1, 79-88; Siegrist & Eberle, Trends Endocrinol. Metabol. 1995, 6, 115-120). Recent data also indicate that the MC1-receptor is expressed in limited areas (periaqueductal gray) of the rat and human brains (Xia et al., Mol. Neurosci. 1995, 6, 2193-2196), as well as in the testis (Vanetti et al., FEBS Lett. 1994, 348, 268-272). Also, very recently the MC1-receptor was shown to be present on macrophages (Star et al., Proc. Natl. Acad. Sci. USA. 1995, 92, 8016-8020), neutrophils (Catania et al., Peptides. 1996, 17, 675-679), glioma cells and astrocytes (Wong et al., Neuroimmunomodulation. 1997, 4, 37-41), monocytes and endothelial cells (Hartmeyer et al., J. Immunol. 1997, 159, 1930-1937, and references therein). Evidence was first found that the MC5-receptor was expressed in brain

and skeletal muscle as well as with lower levels in retina, lung, testis, spleen, heart, kidney, and liver (Chhajlani et al., Biochem. Biophys. Res. Commun. 1993, 195, 866-873; Fathi et al., Neurochem. Res. 1995, 20, 107-113). More recent studies have indicated that it is also present in exocrine glands (van der Kraan et al., Endocrinol. 1998, 139, 2348-2355). Using RT-PCR techniques evidence was also found for the expression of the different MC-receptors in various tissues (Chhajlani et al., Biochem. Mol. Biol. Int. 1996, 38, 73-80).

However MSH-receptors have been known as physiological entities since 1957. Binding sites for MSH/ACTH peptides were identified in a number of brain and peripheral tissues (Hnatowich et al., Can. J. Physiol. Pharmacol. 1989, 67, 568-576; Tatro & Reichlin, Endocrinology 1987, 121, 1900; Lichtensteiger et al., Ann. N. Y. Acad. Sci., 1993, 680, 652-654; Tatro & Entwistle, Brain Research 1994, 635, 148). Peptide structure-activity studies of these receptors have been performed on melanophores from lower vertebrates like Rana pipiens (frog), Anolis carolinensis (lizard) and Xenopus laevis (toad). Receptor studies were later also performed by binding on melanoma cell lines (Eberle et al., J. Recept. Res. 1991, 11, 311-322). These test systems gave comparable results and it is now known that the data obtained with these systems refer to the MC1-receptor.

There appear to exist distinct relationships between the MC-receptors and the genetic locus agouti, the latter which is involved in the control of the relative amounts of eumelanin (brown-black) and phaeomelanin (yellow-red) pigments in mammals. The agouti locus encodes a 131-amino-acid protein which is produced in the hair follicle and which acts on follicular melanocytes to inhibit α-MSH-induced eumelanin production resulting in different

colours in mammalian fur, an effect which has been attributed to an antagonistic action of the agouti protein on MC1-receptors (Lu et al., Nature. 1994, 371, 799-802).

Ectopic expression of agouti occurs in particular strains of obese mice, e.g. the lethal yellow (Ay) mouse, and it is well known that the ubiquitous unregulated expression of agouti is associated with both yellow fur and obesity (see Perry et al., Genetics 1995, 140, 267-274, and references therein). A further very strong link with MC-receptors, agouti and control of feeding was provided in 1994 with the discovery that the agouti protein besides being an antagonist at MC1 was also an antagonist at MC4-receptors (Lu et al., Nature. 1994, 371, 799-802). In these tests of Lu et al., α-MSH was shown to increase cAMP in cells transfected with either MC1, MC3, MC4 and MC5 receptors. Only in the MC1 and MC4-receptor expressing cells did low concentrations of agouti (0.7 nM) cause a parallel shift of the α-MSH dose-effect curve to the right without affecting the maximal response level of α-MSH thus clearly showing a competitive antagonistic action of agouti on MC1 and MC4-receptors. By contrast the cAMP stimulatory action of α-MSH, that could be induced in MC3 and the MC5-receptor expresing cells, was not blocked by agouti (Lu et al., Nature. 1994, 371, 799-802). The K_b-value (i.e. blocking dissociation constant) of agouti for the MC4-receptor that is possible to estimate from the data provided by Lu et al. is 1.2×10^{-10} M. For the MC1-receptor the K_b value was 3.2×10^{-10} M. For the MC3-receptor 0.7 nM agouti was completely ineffective and for the MC5-receptor even 100 nM of agouti was tested and found to be ineffective. Thus, these data show clearly that agouti is a very strong competitive antagonist at MC1 and MC4-receptors. From these studies Lu et al. stated that "because agouti

also antagonizes MC4-R function, ectopic overexpression of agouti may lead to obesity in the lethal yellow mouse (*AY*) through pathological antagonism of melanocortin receptor(s) expressed outside the hair follicle" (Lu et al., Nature. 1994, 371, 799-802).

Moreover, along this line Blanchard et al. (Biochemistry 1995, 34, 10406-10411) had found that agouti induced a strong competitive antagonistic action of the action of MSH-peptides on MSH-receptors in melanoma cells, the K_i (i.e. MSH-receptor binding dissociation constant) of agouti amounting to 0.3 nM, and therefore stated that the phenotypic changes observed in agouti mice such as obesity and hyperinsulinemia might be due to direct action of agouti at novel melanocortin receptor(s).

In the past intense efforts were made to devise synthetic agents having either agonistic or antagonistic activity at MSH-receptors. Numerous linear and cyclic peptides have been synthesized that showed varying capacities to bind to and to activate or block the MSH-receptors. The early studies concentrated on the effects of such peptides on MSH-receptors located on melanophores and melanocytes and led to the development of various MSH-peptide analogues with agonistic activities, including what were called "melanotropic super agonist analogues" (see e.g. De Wied and Wolterink, Ann. N Y Acad. Sci. 1988, 525, 130-140; Eberle, AN: The melanotropins: Chemistry, physiology and mechanisms of action. Basel: Karger, Swizerland. 1988, ISBN 3-8055-4678-5; Sawyer et al., Peptide Research 1989, 2, 140-146 and references therein). Among the numerous linear peptides that were synthesized may be mentioned [Nle^4,D-Phe^7]αMSH (Melanotan-I; NDP-MSH) which had enhanced melanotropic activity on amphibian melanophores and the capacity to stimulate cAMP in mouse melanoma cells (Sawyer et al., J.

Med. Chem. 1982, 25, 1022-1027). Many cyclic peptide analogues have also been synthesized, e.g. those containing disulphide bridges (see e.g. Cody et al., in The Melanotropic Peptides, vol III (ed. ME Hadley), CRC Press, Boca, Raton, Florida, 1988, p. 75-92: US 4,485,039) or lactam bridges (Al-Obeidi et al., J. Med. Chem. 1989, 32, 2555-2561).

The first synthetic antagonistic peptide compounds for MSH-receptors was accomplished by the provision of some [His^1,Lys^6]hexapeptides which were capable with low potency of blocking the effect of MSH-peptides on frog melanophores (Sawyer et al., Peptide Research 1989, 2, 140-146). Using a similar approach a more potent antagonist (Ac-Nle-Asp-Trp-D-Phe-Nle-Trp-Lys-NH_2, SEQ ID NO: 1) for MSH-receptors in frog skin melanophores was found (Al-Obeidi et al., Int. J. Peptide Protein Res. 1990, 35, 228-234).

Later on the cloning of the five different MC-receptors allowed the assay of substances separately on each of the MC-receptor subtypes. Thus, e.g. assays using radioligand binding or cAMP measurements in cells artificially expressing the various MC-receptors have been described (WO 93/21316; WO 94/04674; US 5,622,860; Schiöth et al., Eur. J. Pharmacol., Mol. Pharm. Sect. 1995, 288, 311-317; Schiöth et al., Pharmacol. Toxicol. 1996, 79, 161-165) which could be used to assess the pharmacological activities of substances on the MC-receptors. When such tests were applied it was found that the natural MSH-peptides as well as most of the previously developed MSH-peptide analogues showed a potency order for the MC-receptors: MC1 > MC3 > MC4 > MC5 (Schiöth et al., Eur. J. Pharmacol., Mol. Pharm. Sect. 1995, 288, 311-317; Schiöth et al., Pharmacol. Toxicol. 1996, 79, 161-165). However, a few compounds have emerged that are claimed to show

selective actions on other MC-receptors. Thus, the data of Adan et al. (Eur. J. Pharmacol. 1994, 269, 331-337) suggested that some linear ACTH(4-10) peptides were weakly selective antagonists at MC4-receptors. Some cyclic lactam peptides have also been described which were claimed to show selectivity and antagonistic activity for MC-receptor subtypes (Hruby et al., J. Med. Chem., 1995, 38, 3454-3461; US 5,731,408). More recently, some MC4-receptor selective 26 and 29-membered antagonistic cyclic peptides were also described (Schiöth et al., Br. J. Pharmacol, 1998, 124, 75-82; WO98/37097).

Conformationally constrained α-MSH analogues with specific central nervous system actions have also been described (US 4,649,191), as well as bicyclic α-MSH analogues (Haskell-Luevano et al., J.Med. Chem 1995, 38, 1736-1750).

Moreover some ring closed small cyclic peptides related to MSH-peptides have been described in Schiöth et al., Eur. J. Pharmacol, 1997, 319, 369-373. This paper does not discuss the biological uses of the peptides referred to therein.

The role of MC-receptors for control of food intake has more recently attracted much attention in a number of recent studies. These studies have verified that agonistic action on MC-receptors are related to decrease in food intake whereas the antagonistic actions have the opposite effect. In particular the possibility that the MC4-receptor is of importance in these effects have attracted much attention (Fan et al, Nature. 1997, 385, 165-168; Huszar et al., Cell. 1997, 88, 131-141; Chagnon., et al., Mol. Med. 1997, 3, 663-673; Fruedman, Nature, 1997, 385, 119-120; Kask et al., Biochem. Biophys. Res. Commun. 1998, 245, 90-93; WO 97/47316;

WO98/10068). There is also genetic evidence for roles of MC-receptors in regulation of body weight as genetic variants of both the MC4 and MC5-receptor genes were found to be related to obesity phenotypes in a human population (Chagnon et al., Mol. Med. 1997, 3, 663-673). Interestingly, this linkage with human obesity phenotypes was found to be strongest for the MC5 receptor. The MC5 receptor is expressed in both the central nervous system and in adipose tissue which thus indicates important roles for the MC5 receptor in body weight homeostasis (Chagnon et al., Mol. Med. 1997, 3, 663-673).

From a physiological point of view α-MSH is otherwise mainly known for its ability to regulate peripheral pigmentation (Eberle, AN: The melanotropins: Chemistry, physiology and mechansims of action. Basel: Karger, Swizerland. 1988, ISBN 3-8055-4678-5), whereas ACTH is known to induce steroidoneogenesis (Simpson and Waterman, Ann. Rev. Physiol., 1988, 50, 427-440). These effects are clearly now known to be mediated by, respectively, the MC1 and MC2-receptors. However, MC-receptors are also linked to a variety of other physiological actions thought to be mediated by distinct subtypes of the MC-receptors, but in many cases it is not entirely clear which one of the subtypes is responsible for the effect.

It has also long been known that MSH-peptides may affect many diverse processes such as motivation, learning, memory, behaviour, inflammation, body temperature, pain perception, blood pressure, heart rate, vascular tone, brain blood flow, nerve growth, placental development, aldosteron synthesis and release, thyroxin release, spermatogenesis, ovarian weight, prolactin and FSH secretion, uterine bleeding in women, sebum and pheromone secretion, blood glucose levels, intrauterine foetal growth, as well as other events surrounding

parturition (Garrud et al., Physiol. Psychol. 1974, 112, 109-119; Wiegant et al., Life Sci. 1979, 25, 1791-1796; O´Donahue, et al., Peptides. 1981, 2, 101-104; O'Donahue and Dorsa, Peptides. 1982, 3, 353-395; De Wied and Jolles, 1982, Physiol. Rev. 62, 976; Klein et al., Life Sciences. 1985, 36, 769-775; Feng et al., Brain Res. 1987, 18, 473-477; Eberle, AN: The melanotropins: Chemistry, physiology and mechanisms of action. Basel: Karger, Swizerland. 1988, ISBN 3-8055-4678-5; Gruber, and Callahan, Am. J. Physiol. 1989, 257, R681-R694; De Wildt et al., J. Cardiovascular Pharmacology. 1995, 25, 898-905), as well as they are capable of inducing natriuresis (Lin et al., Hypertension. 1987, 10, 619-627).

The MC5-receptor has recently been attributed a role in control of exocrine gland function (van der Kraan, et al., Endocrinol. 1998, 139, 2348-2355; Chen et al., Cell. 1997, 91, 789-798).

In addition the melanocortic peptides have distinct effects on sexual functions in that melanocortic peptides cause erection in males (Donovan, Psychol. Med. 1978, 8, 305-316), an effect presumed to be mediated by a central agonistic effect of the peptide on MC-receptors. The capacity of the MC-receptor agonist MT-II to induce erection is also described (Wessells and Fuciarelli, J. Urol. 1998, 160, 389-393).

MSH-receptors are thought to have roles in modulation of the immune system and in modulation of inflammation both in the periphery and in the central nervous system (see Star et al., Proc. Natl. Acad. Sci. USA. 1995, 92, 8016-8020; Bhardwaj et al., J. Immunol. 1996, 156, 2517-2521; Catania et al., Peptides. 1996, 17, 675-679; Goninard et al., Pigment Cell Res. 1996, 9, 148-153; Rajora et al.,

J. Neurosci. 1997, 17, 2181-2186; Rajora et al., Peptides. 1997, 18, 381-385; Lipton and Catania, Immunology Today. 1997, 18, 140-145; Wong et al., Neuroimmunomodulation. 1997, 4, 37-41; Luger et al., J. Invest. Dermatol. Symp. Proc. 1997, 2, 87-93; Hartmeyer et al., J. Immunol. 1997, 159, 1930-1937).

Important aspects of these antiflammatory actions are related to effects on nitric oxide (NO) metabolism. α-MSH was shown to inhibit formation of nitric oxide in cultured murine macrophages stimulated with bacterial lipopolysaccharide and γ-interferon, an effect claimed to be caused by the inhibition of the production of NO synthase (NOS) by the stimulation of MC1-receptors in macrophages (Star et al., Proc. Natl. Acad. Sci. USA. 1995). As NO is believed to be a common mediator of all forms of inflammation this indicates that stimulation of MC1-receptors mediates the anti-inflammatory effect earlier known to be induced by MSH-peptides.

α-MSH is also known to increase the formation of interleukin 10 (IL-10) in monocytes, which is believed to be an important component in immunosuppressive effects induced by α-MSH (Bhardwaj et al., J. Immunol. 1996, 156, 2517-2521).

Recent studies also show that α-MSH potently inhibits the chemotactic migration of neutrophils (Catania et al., Peptides. 1996, 17, 675-679). Moreover, neutrophils were shown to contain MC1-receptor mRNA, which was upregulated on stimulation of the neutrophils with interferon and bacterial lipopolysaccharide (Catania et al., 1996, ibid.). Thus, as neutrophil migration constitutes an important component in early inflammation, these results again indicate the importance of the MC1-receptor as a mediator of the inhibition of inflammation.

In another study, the injection of α-MSH, as well as the MSH-analogue [Nle^4, D-Phe^7]- α-MSH (NDP-MSH) was shown to inhibit the release of cytokines IL-1 and TNF-α into the blood after intra-peritoneal injection of lipopolysaccharide (Goninard et al., Pigment Cell Res. 1996, 9, 148-153). This supports the anti-inflammatory role of MSH-peptides.

Important anti-inflammatory roles of MC-receptors (presumed to be of the MC1-type) have also been implicated in the brain since α-MSH inhibits the production of tumour necrosis factor alpha (TNF-α) *in vivo*, as well as *in vitro* on glioma cells; in the later case α-MSH was shown to inhibit formation of TNF-α induced by bacterial endotoxin (Wong et al., Neuroimmunomodulation., 1997, 4, 37-41). In another study α-MSH given intracerebroventricularly or intraperitonally inhibited formation of central TNF-α induced by locally administered bacterial lipopolysaccharide (Rajora et al., J. Neurosci. 1997, 17, 2181-2186.). TNF-α occurs in neurological disorders, infection and injury of the brain, and is thought to underlie pathological processes in the brain. These data indicate an important role of MC-receptors as mediators of central anti-inflammatory actions.

Recently α-MSH was also shown to reduce inflammation in a model for inflammatory bowel disease (Rajora et al., Peptides. 1997b, 18, 381-385).

The α-MSH peptide too is ascribed an important role in cutaneous biology. Most well known is its ability to stimulate pigment formation of the skin. However, α-MSH may act not only on MC-receptors located on melanocytes but also on immunocompetent and inflammatory cells,

keratinocytes, fibroblasts and endothelial cells of the skin, thereby modifying keratinocyte proliferation and differentiation, and regulating endothelial cell and fibroblast cytokine production, as well as fibroblast collagenase production. α-MSH is known to down-regulate the production of pro-inflammatory cytokines and accessory molecules on antigen presenting cells. In contrast suppressor factors such as IL-10 are upregulated by α-MSH (Luger et al., J. Invest. Dermatol. Symp. Proc. 1997, 2, 87-93). *In vivo* data show that systemic application of α-MSH inhibits the induction and elicitation of contact-hypersensitivity and induces hapten tolerance (Luger et al., J. Invest. Dermatol. Symp. Proc. 1997, 2, 87-93). Thus, the accumulating evidence indicates that the stimulation of MC-receptors, presumably of the MC1-receptor subtype, mediates important negative regulation mechanisms of cutaneous inflammation and hyper-proliferative skin diseases (Luger et al., J. Invest. Dermatol. Symp. Proc. 1997, 2, 87-93).

In addition to these findings, Hartmeyer et al. (J. Immunol. 1997, 159, 1930-1937) have shown that α-MSH increases MC1-receptor expression in dermal microvasculature endothelial cells and causes increased release of interleukin 8 (IL-8) from these cells. This indicates a role of MC1-receptors in the skin as modulators of inflammation and immunity (see Hartmeyer et al., J. Immunol. 1997, 159, 1930-1937).

For further reading on the anti-inflammatory role of MSH peptides reference is made to the review by Lipton and Catania (Immunology Today. 1997, 18, 140-145).

There is also strong evidence that the melanocortic system is involved in drug addiction. Thus, it has been noted that MSH peptides antagonize opiate tolerance and

dependence (Szekely et al., Life Sci. 1979, 24, 1931-1938; Contreras & Takemori, J. Pharmacol. Exp. Ther., 1984, 229, 21-26). More recently it has been shown that MC4-receptor mRNA, as well as MSH-receptor binding activity, was reduced in several brain areas, namely the striatum, periaqueductal gray, nucleus accumbens and olfactory turbercle, that are related to the effects of opiates and their addictive effects, after the chronic administration of morphine (Alvaro et al., Mol. Pharm., 1996, 50, 583-591). It was also stated that similar effects on MC-receptors could be seen after chronic administration of cocaine (Alvaro et al., Life Sci, 1997, 61, 1-9). It was speculated that there existed a balance in MSH receptor and opiate receptor induced effects in the brain, stimulation of the former leading to increase in cAMP and stimulation of the latter a decrease in cAMP. The acute opiate induced state (i.e. after administration of morphine) is related to the inhibition of cAMP formation leading to changes in cellular processes. After chronic administration of opiates a downregulation of MC-receptors may lead to further decrease in cAMP which, however, may be counteracted by adaptive increase in adenylatecyclase and protein kinase A activities. If opiates are withdrawn it was speculated that the observed withdrawal symptoms are due to overactivation of cAMP both via absence of opiate receptor stimulation and increased MC-receptor activation (Alvaro et al., Life Sci, 1997, 61, 1-9). It is conceivable that both MSH-receptor stimulation and MSH-receptor blockade could have profound effects on the morphine and other addictive states (e.g. cocaine, alcohol, amphetamine and other narcotics), which effects could be beneficial in treatments of addiction to such agents. Both an MC-receptor agonist or an MC-receptor antagonist could be useful depending on what treatment effect is desired (e.g. prevention of addiction, reduction of withdrawal

symptoms, elimination/reduction of reward effects caused by a morphine, cocaine, amphetamine, alcohol and other narcotics).

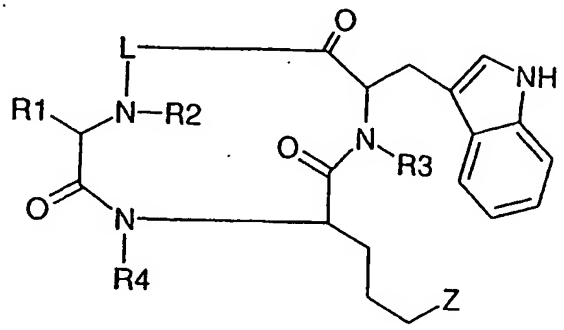
MSH peptides are also known to have both neurotrophic and myotrophic actions and have been suggested to be effective in treatment of various muscular diseases such as degenerative myopathies of either pure or mixed origin, such as muscular dystrophy, infantile spinal atrophy, and hypotonia (see Strand et al., Peptides. 1993, 14, 287-296 and references therein). They are also said to improve recovery in spinal cord injury (van de Ment et al., Neurosurgery. 1997, 40, 122-131).

There is therefore a need to provide means which may be used to regulate food intake, weight homeostasis and growth of an animal, in particular a mammal, preferably a human. Such means may be provided by compounds that bind to melanocortin receptors, thereby causing an agonistic or an antagonistic action on the receptor.

There is further a need to provide compounds that may bind selectively to different MC-receptor types, such as the MC4-receptor. Such compounds, by virtue of their capacity to bind to MC-receptors, may be useful in treating conditions in animals, mammals and/or humans which involve MC-receptors. It is desired to provide such compounds which may be capable of penetrating the blood brain barrier, as well as optionally being absorbed systemically after oral administration.

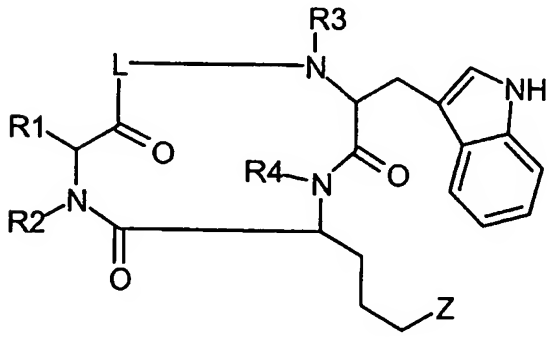
It would also be desirable to provide a compound showing a rigid three dimensional structure which can be determined in water solution by the use of NMR-techniques and to provide means for use of said structure in the design of novel compounds.

According to one aspect, the present invention relates to compounds having the general formula (1)



(1)

and/or compounds having the general formula (2):



(2)

and the uses thereof.

L is a linking group so as to create a cycle which contains from 18 to 21 ring-atoms, with 20 ring-atoms being preferred. Preferably, L should contain a disulphide bridge, the 2 connected sulphur atoms in this bridge being part of the ring.

Z is selected from $-NH_2$, $-CH_2NH_2$ and guanidino, with guanidino being preferred.

R1 is selected from X and $-CH_2X$ where X is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted

heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl or a functional group.

Preferably R1 does not represent benzyl or 4-hydroxybenzyl or 1H-indol-3-yl.

More preferably R1 is $-CH_2X$ where X is selected from phenyl substituted with halogen, methyl, phenyl, methoxy, nitro, preferably in the 3 and/or 4 position, or 2-naphthyl or an aromatic system consisting of 3 fused benzene rings.

Most preferably, R1 is $-CH_2X$ where X is 2-naphthyl.

In some preferred embodiments of the invention, the number of atoms in X exceeds 11, more preferably exceeds 12, even more preferably exceeds 13, still even more preferably exceeds 14, even still more preferably exceeds 15 and most preferably exceeds 16.

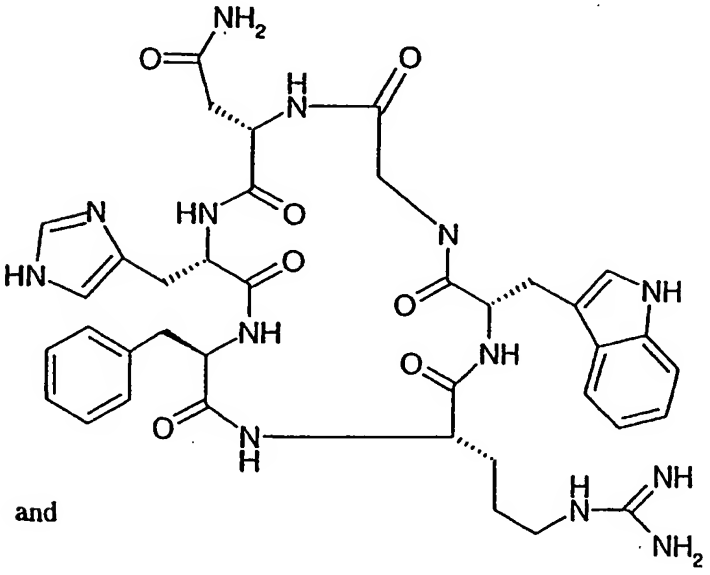
In some preferred embodiments of the invention, the number of carbon atoms in X exceeds 6, more preferably exceeds 7, even more preferably exceeds 8, still even more preferably exceeds 9, even still more preferably exceeds 10 and most preferably exceeds 11.

In some preferred embodiments of the invention, the number of heavy atoms in X exceeds 5, more preferably exceeds 6, even more preferably exceeds 7, still even more preferably exceeds 8, even still more preferably exceeds 9 and most preferably exceeds 10.

In some preferred embodiments of the invention, the mass of X exceeds 77.3 daltons, and even more preferably exceeds 79.9 daltons.

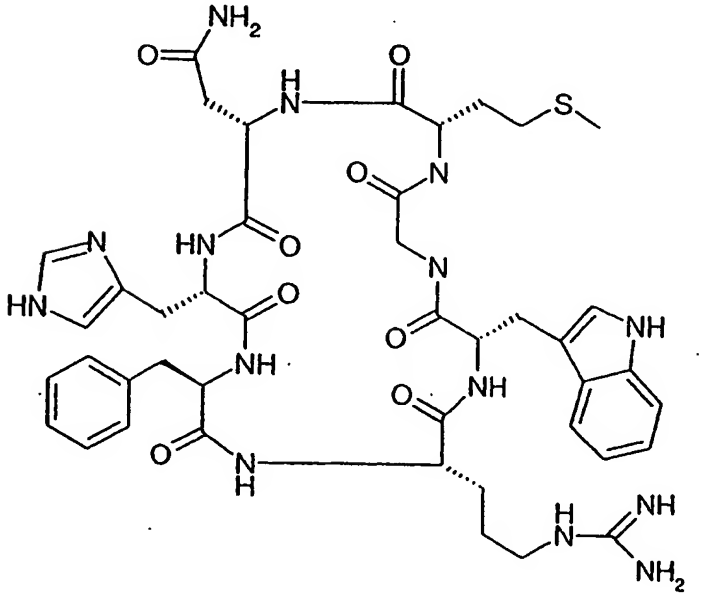
R2, R3 and R4 are selected from hydrogen and methyl, with hydrogen being preferred.

The compounds cNHdFRWG (SEQ ID NO: 2) and cMNHdFRWG (SEQ ID NO: 3) having structural formulae as follows



cNHdFRWG

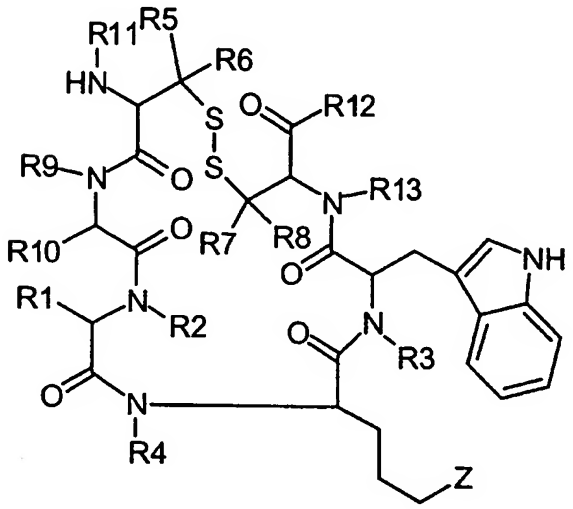
and



cMNHdFRWG

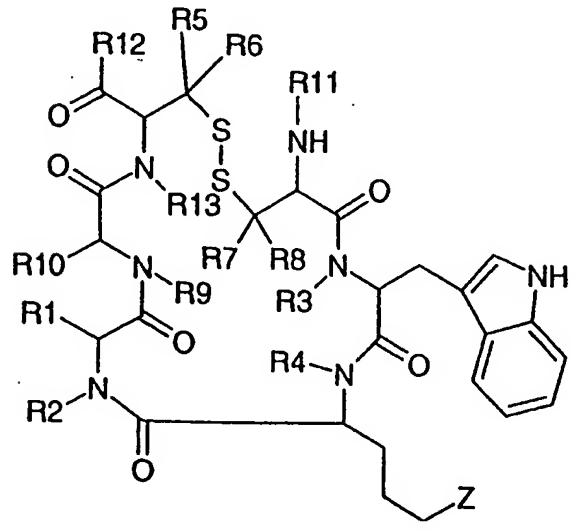
are specifically excluded from the scope of definitions of compounds 1 and 2.

Preferred embodiments of the invention relate to compounds having the general formula (3):



(3)

and compounds having the general formula (4)



(4)

and to uses thereof.

R1, X and Z are as defined above.

R2, R3, R4, R5, R6, R7, R8, R9 and R13 are selected from hydrogen and methyl, with hydrogen being preferred.

R10 is selected from X, or $-CH_2X$ where X is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, or a functional group.

R10 is preferably H or methyl.

In some preferred embodiments of the invention R10 is selected so as to have less than 12 atoms, more preferably less than 11 atoms, even more preferably less than 10 atoms, still even more preferably less 7 than atoms.

In some preferred embodiments of the invention R10 is selected so as to have less than 5 carbon atoms, more preferably less than 4 carbon atoms, even more preferably less than 3 carbon atoms and most preferably less than 2 carbon atoms.

In some preferred embodiments of the invention R10 is selected so as to have less than 5 heavy atoms, more preferably less than 4 heavy atoms, even more preferably less than 3 heavy atoms and most preferably less than 2 heavy atoms.

In some preferred embodiments of the invention R10 is selected so as to have a mass of less than 82 daltons, more preferably less than 81 daltons, even more preferably less than 78 daltons, still even more preferably less than 74 daltons, even still somewhat more preferably less than 56 daltons, and even more preferably less than 44 daltons and most preferably less than 30 daltons.

R10 is most preferably hydrogen or methyl.

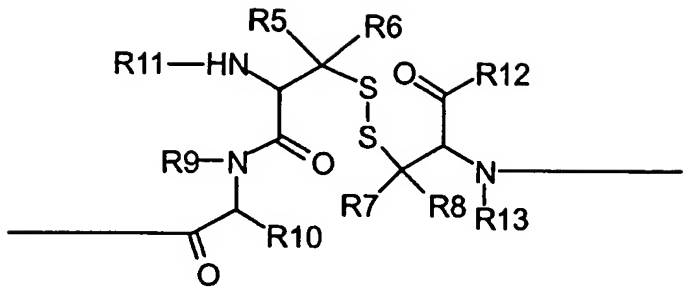
R11 is selected from H, acetyl, alkyl, amino-acid residue, amino-acid analogue residue, peptide residue and a functional group, with hydrogen or acetyl being preferred.

R12 is selected from hydrogen, $-NH_2$, hydroxy, methoxy, isopropoxy, alkyl, amino-acid residue, amino-acid analogue residue, peptide residue and a functional group, with $-NH_2$ or hydroxy being preferred.

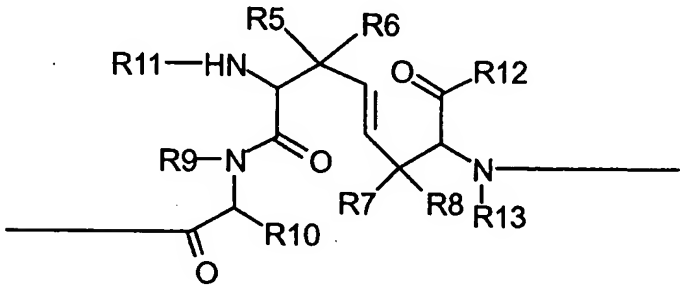
The linking group L is chosen such that it preferably does not affect the ability of the compound to bind to an MSH-receptor. L might have 18, 19, 20 or 21 ring atoms.

Examples of preferred linking groups are given below. In these examples R5 to R13 are defined as given above.

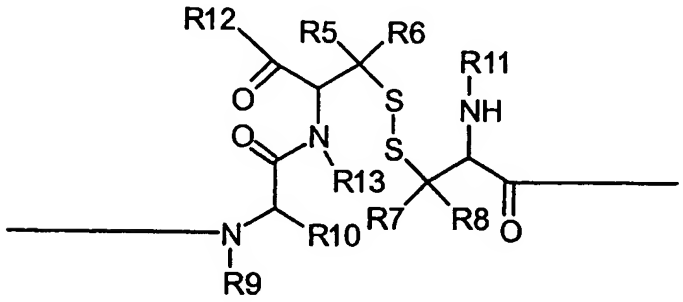
I


R5
R6
R11—HN
S
O
R12
S
R9—N
O
N
R7
R8
R13
R10
O

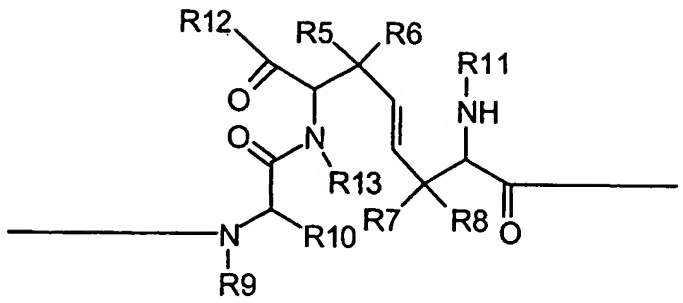
II.


R5
R6
R11—HN
O
R12
R9—N
O
N
R7
R8
R13
R10
O

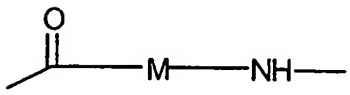
III.


R12
R5
R6
R11
O
S
NH
O
N
S
R13
R7
R8
O
N
R10
R9

IV.

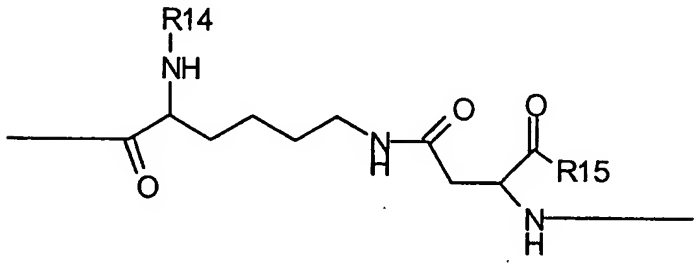


V.



Wherein M is a saturated or unsaturated linear hydrocarbon chain of 7 to 10 carbon atoms.

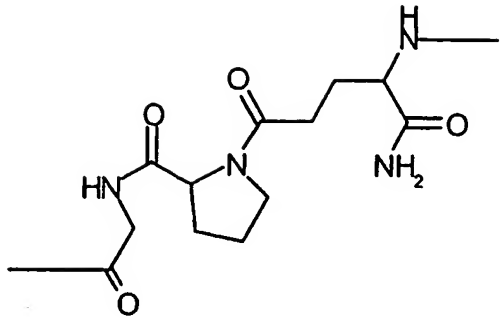
VI.



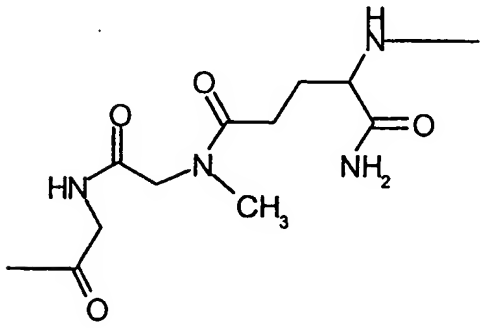
Wherein R14 is selected from hydrogen, acyl, alkyl, amino-acid residue, amino-acid analogue residue, peptide residue and a functional group, with hydrogen or acetyl being preferred.

Wherein R15 is selected from hydrogen, $-NH_2$, hydroxy, alkyl, methoxy, isopropoxy, amino-acid residue, amino-acid analogue residue, peptide residue and a functional group, with $-NH_2$ or hydroxy being preferred.

VII.



VIII.



IX.

The linking group may also comprise other peptide residues, and preferably contains three or four amino acid residues and/or aminoacid analogue residues, the preferred structures being

-Gly-Ala-Gly-

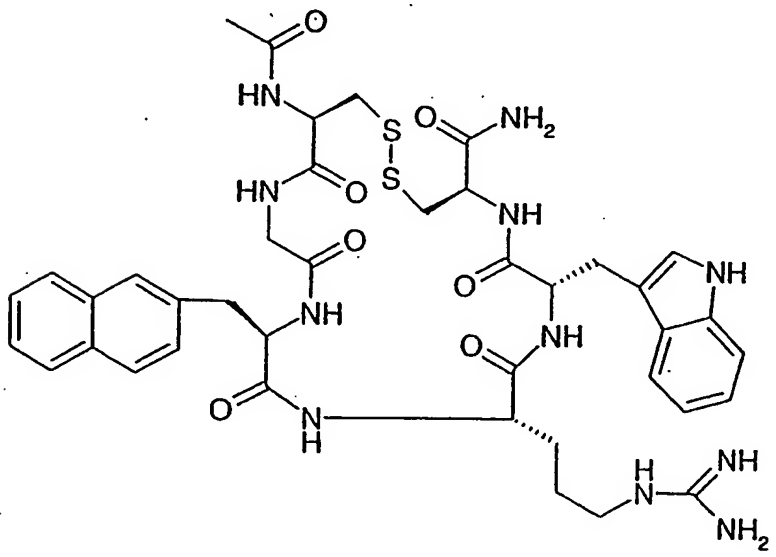
or

-Gly-Gly-Gly-Gly- (SEQ ID NO: 4)

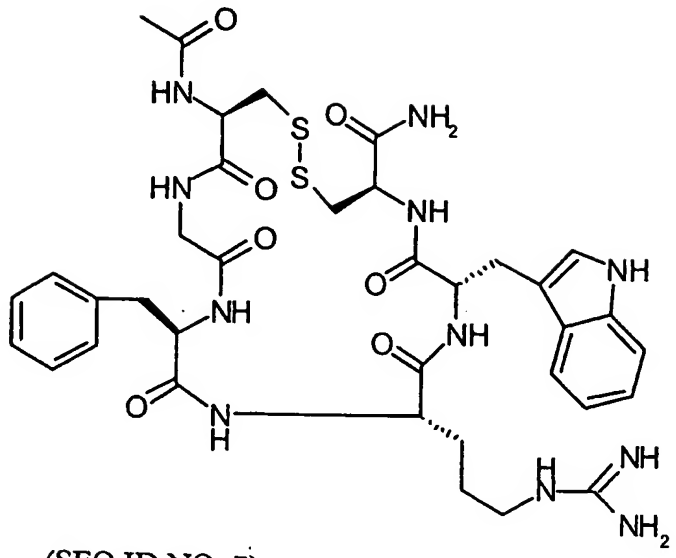
Moreover, the invention refers to all stereoisomeric conformations of the compounds according to formulas (1), (2), (3) and (4). Specific examples of these compounds are shown below.

The invention furthermore refers specifically to compounds Q1 to Q20, having the formulas, respectively:

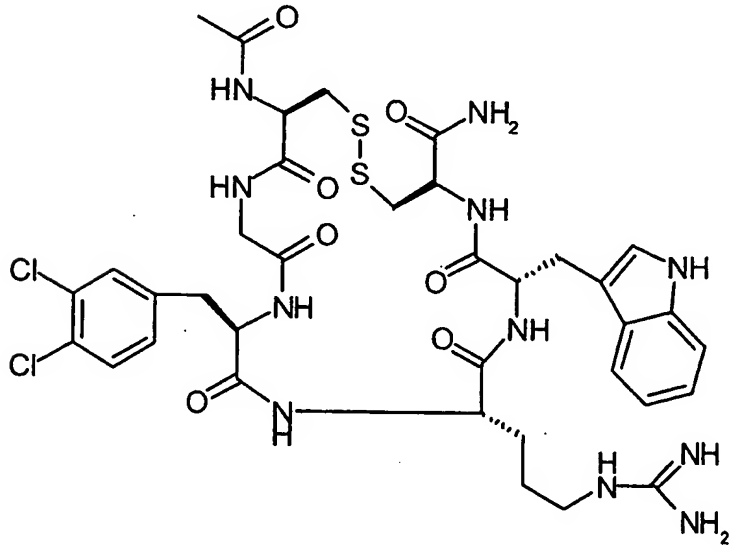
Q1 (SEQ ID NO: 5)



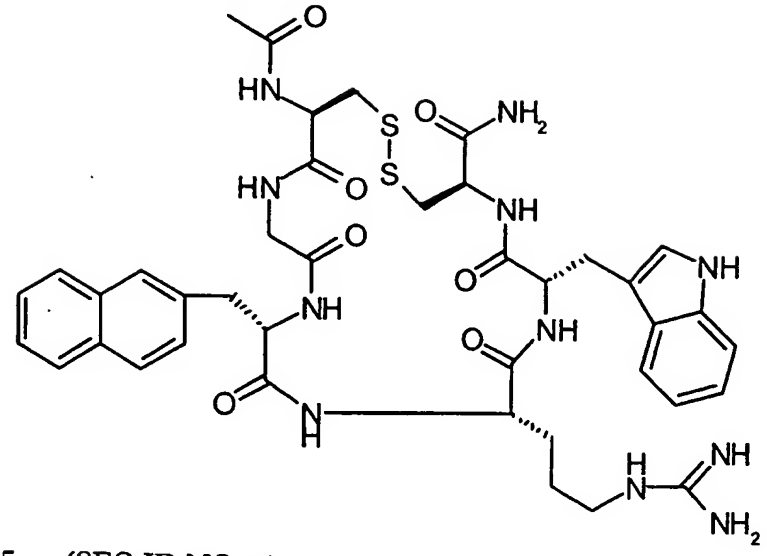
Q2 (SEQ ID NO: 6)



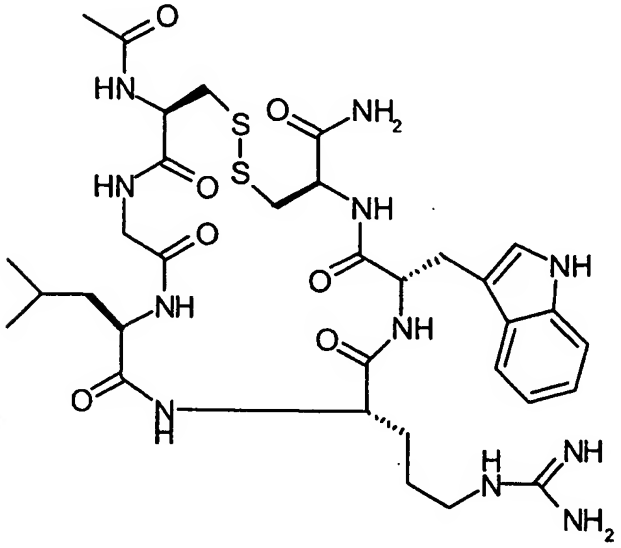
Q3 (SEQ ID NO: 7)



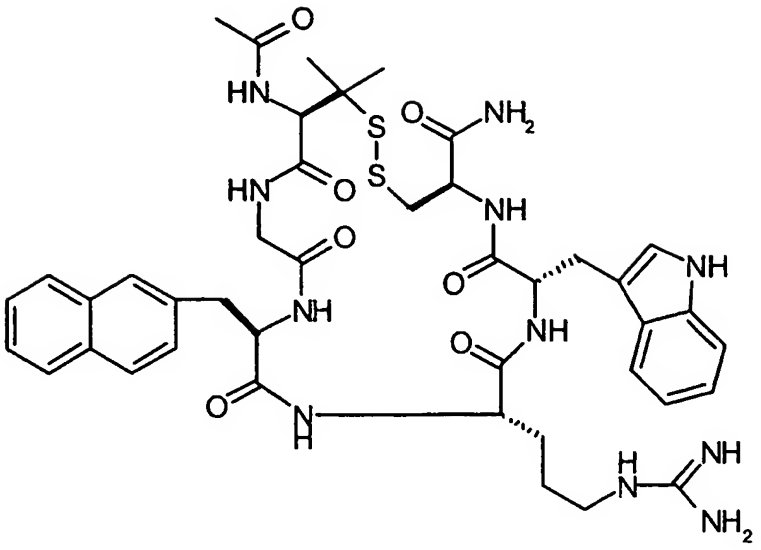
Q4 (SEQ ID NO: 8)



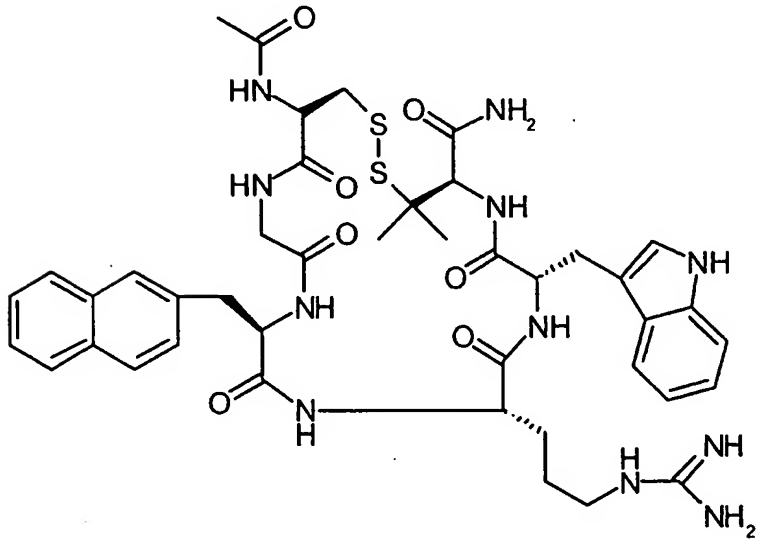
Q5 (SEQ ID NO: 9)



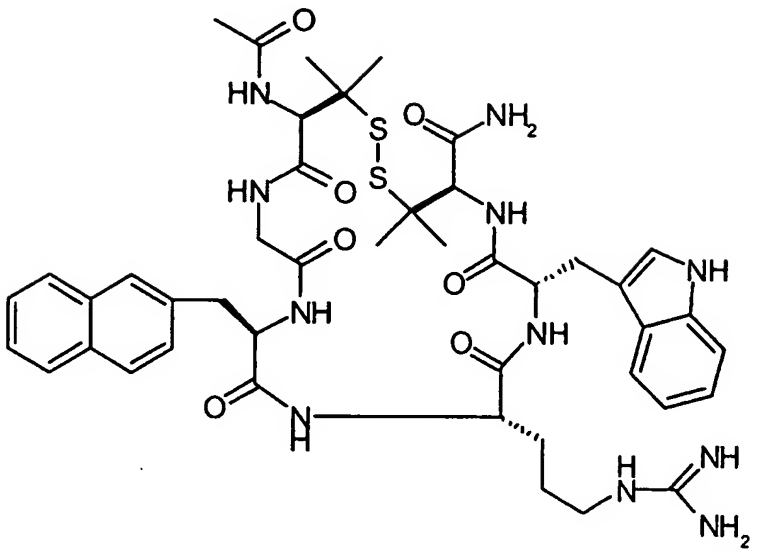
Q6 (SEQ ID NO: 10)



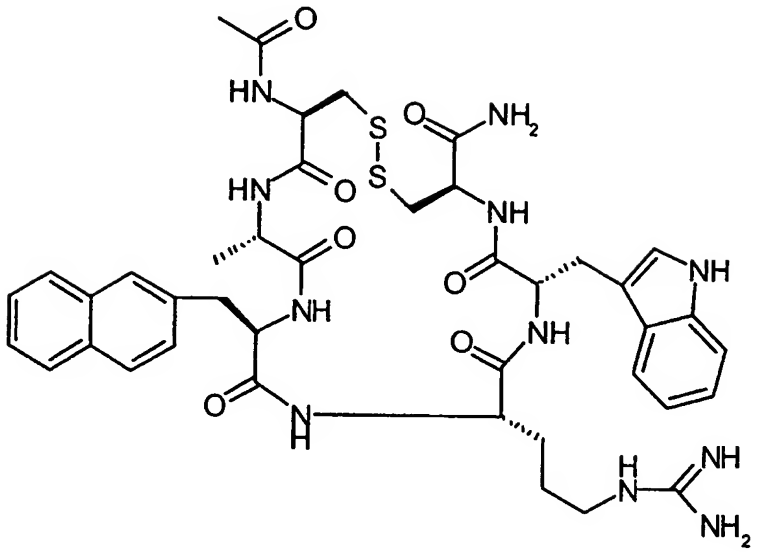
Q7 (SEQ ID NO: 11)



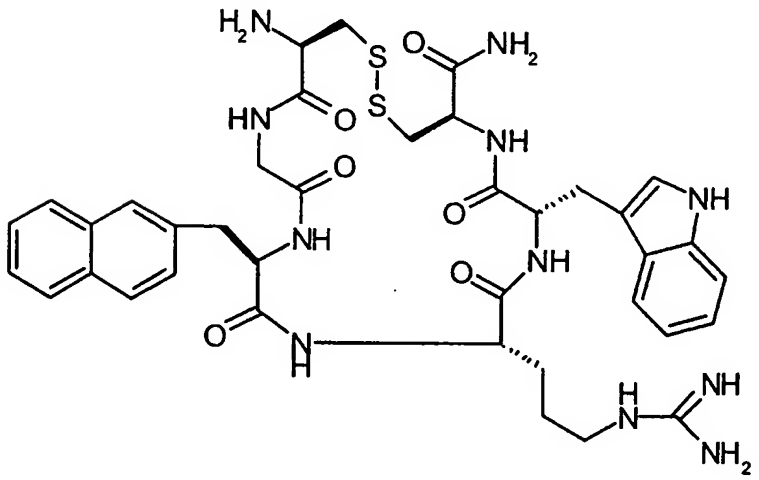
Q8 (SEQ ID NO: 12)



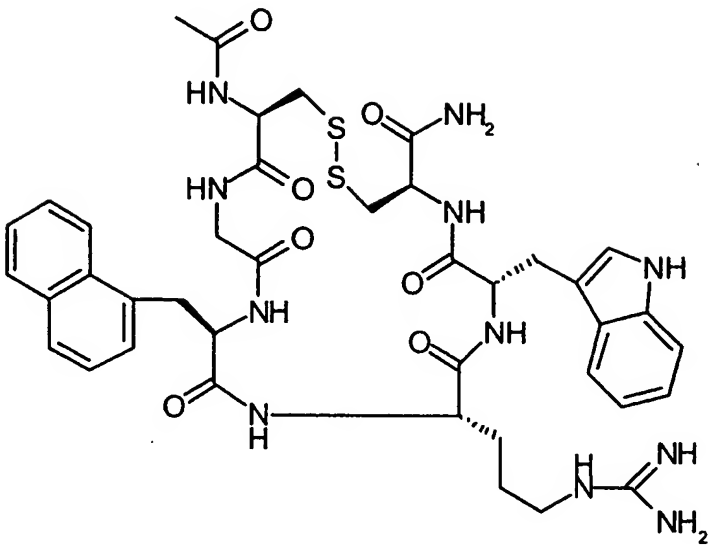
Q9 (SEQ ID NO: 13)



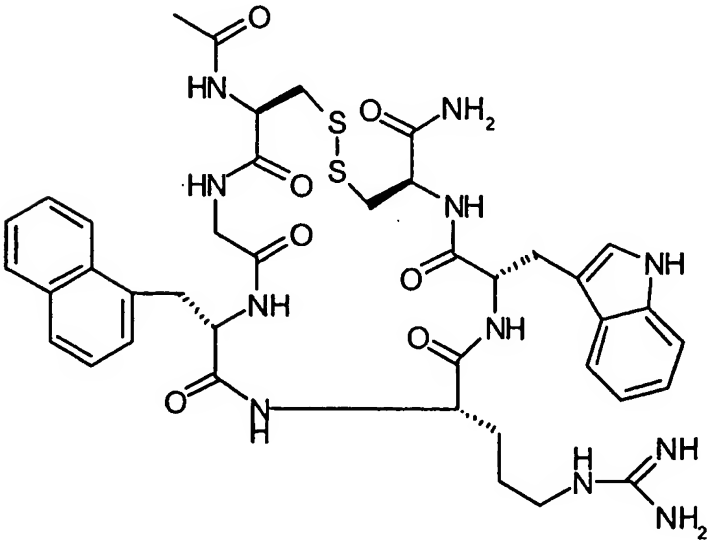
Q10 (SEQ ID NO: 14)



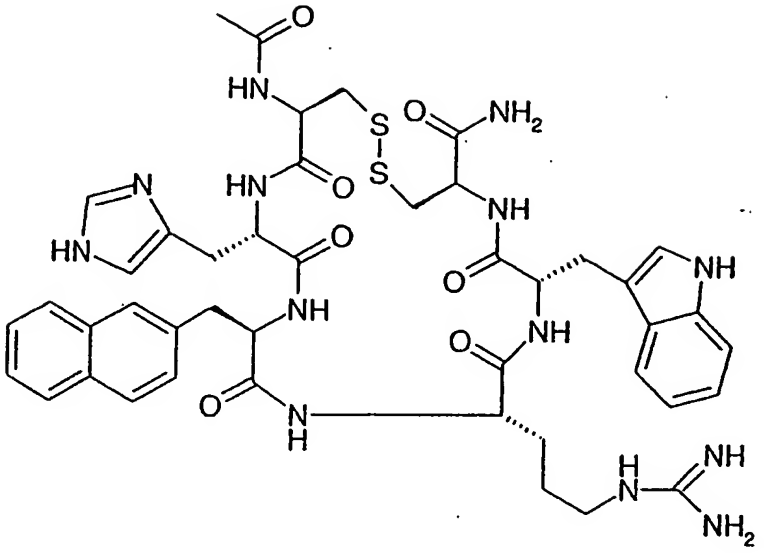
Q11 (SEQ ID NO: 15)



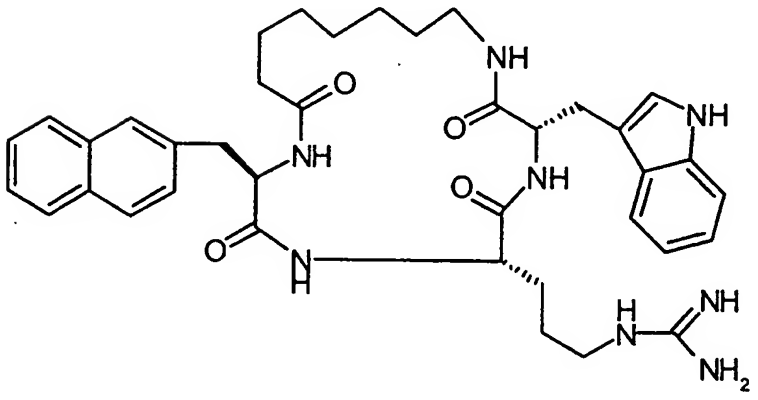
Q12 (SEQ ID NO: 16)



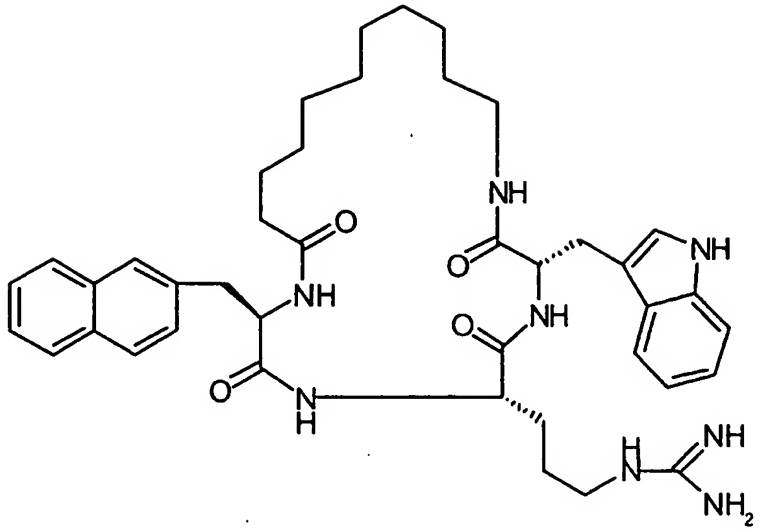
Q13 (SEQ ID NO: 17)



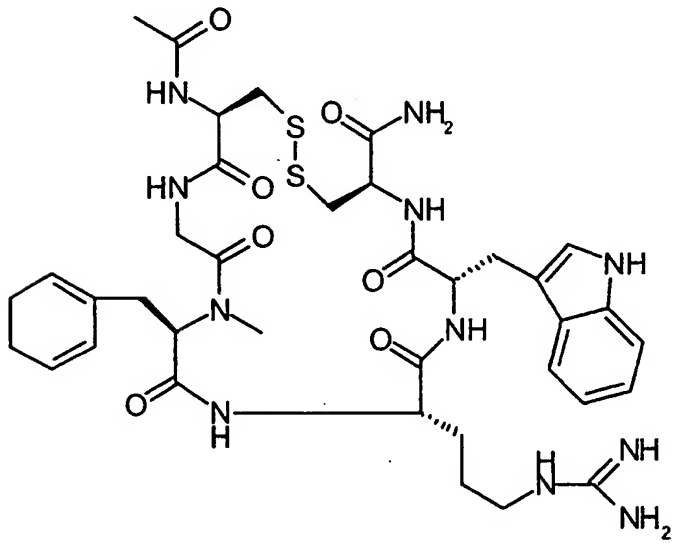
Q14 (SEQ ID NO: 18)



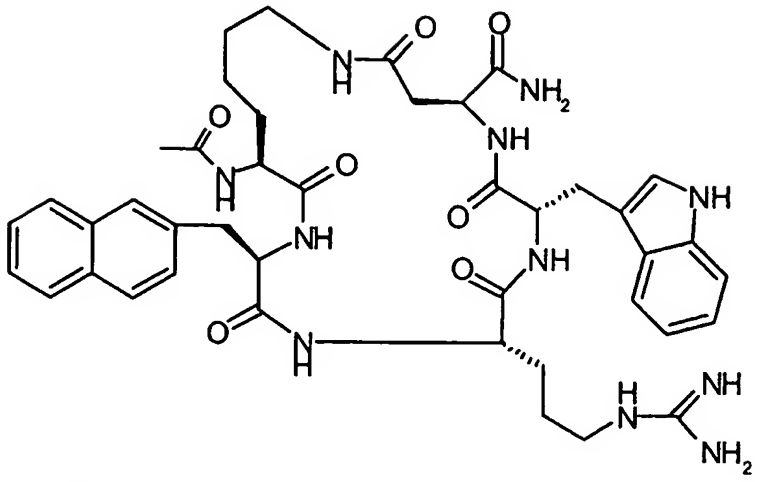
Q15 (SEQ ID NO: 19)



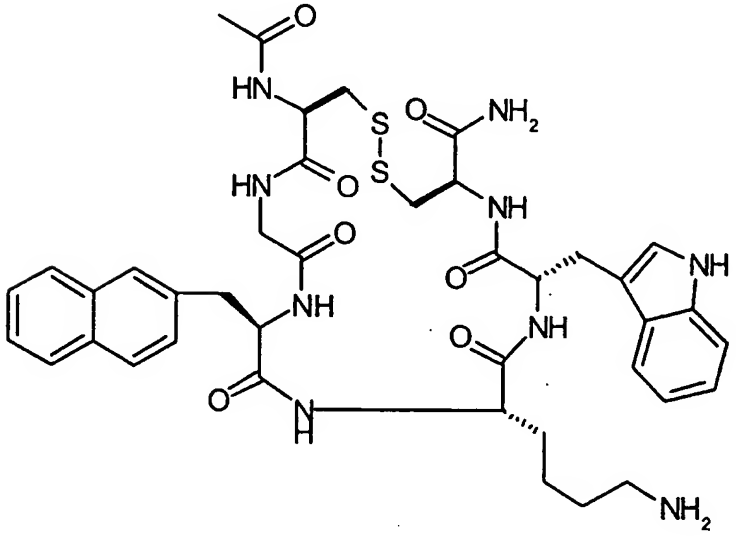
Q16 (SEQ ID NO: 20)



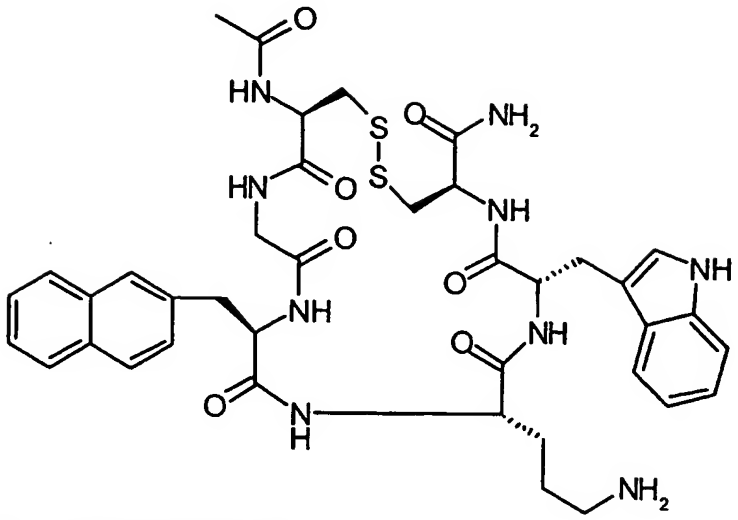
Q17 (SEQ ID NO: 21)



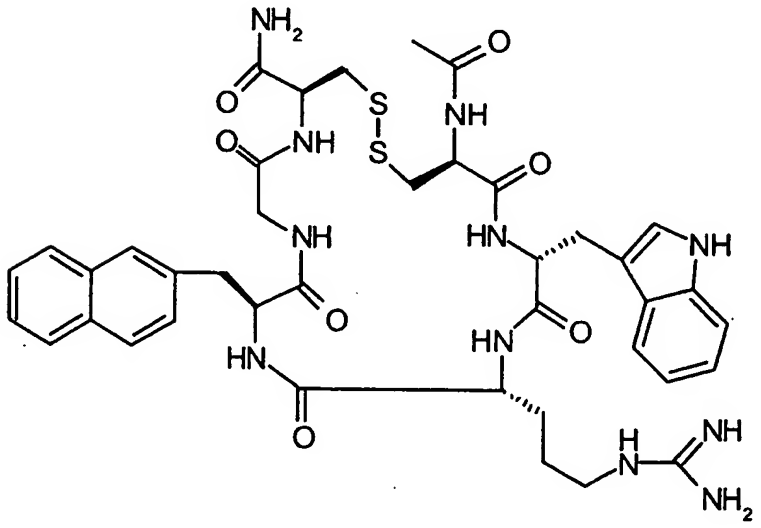
Q18 (SEQ ID NO: 22)



Q19 (SEQ ID NO: 23)



Q20 (SEQ ID NO: 24)



with Compounds Q1 and Q9 being the most preferred.

Included in the invention is a compound of formula (1), (2), (3) or (4) or any of compounds Q1 to Q20 which has been subjected to minor structural alteration by the exchange of one or several hydrogen atoms with methyl. In the preferred embodiment of this aspect of the invention not more than five, preferably not more than four, more preferred not more than three, even more preferred not more than two and most preferred not more than one

hydrogen atom has in said compound been exchanged with methyl. In the most preferred embodiment of the invention said exchange(s) of hydrogen with methyl is made on a hydrogen attached to a nitrogen atom.

Moreover, the atoms forming peptide bonds in the compounds according to formula (1), (2), (3) or (4) or any of compounds Q1 to Q20 may be modified by exchanging carbon, nitrogen or oxygen atoms by other atoms(s), the preferred substitute for carbon being oxygen or sulphur, for nitrogen being carbon and for oxygen being hydrogen or sulphur. In a preferred embodiment of this aspect of the invention preferably less than 5, more preferably less that 4, even more preferably less than 3, still even more preferably less than 2, and most preferably less than 1 of said peptide bond atoms are subjected to said alteration(s).

Included in the invention is also a pro-drug of which after its administration to an animal, mammal or human can form a compound of formula (1), (2), (3) or (4) or any of compounds Q1 to Q20 by metabolism or other chemical reaction(s).

The term "alkyl" as employed herein by itself or as part of another group includes a straight or branched hydrocarbon chain of up to 18, preferably from 1 to 8 carbon atoms, such as methyl, ethyl, propyl, iso-propyl, tert-butyl, butyl, pentyl, hexyl, heptyl, octyl.

The term "heteroalkyl" as employed herein by itself or as part of another group refers to alkyl where one or several carbon atoms are exchanged for a heteroatom.

The term "alkenyl" as employed herein by itself or as part of another group includes a straight or branched

hydrocarbon chain of up to 18, preferably from 2 to 8 carbon atoms comprising one or several carbon-carbon double bonds, such as propenyl, butenyl, pentenyl.

The term "heteroalkenyl" as employed herein by itself or as part of another group refers to alkenyl where one or several carbon atoms are exchanged for a heteroatom.

The term "alkynyl" as employed herein by itself or as part of another group refers to alkyl or alkenyl containing one or several carbon-carbon triple bonds.

The term "heteroalkynyl" as employed herein by itself or as part of another group refers to heteroalkyl or heteroalkenyl containing one or several carbon-carbon triple bonds.

The term "cycloalkyl" as employed herein by itself or as part of another group refers to cyclic hydrocarbons containing from 3 to 12 carbons, preferably 3 to 8 carbons, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and may be fused with 1 or 2 cycles which are independently selected from each other from the group consisting of cycloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, aryl and heteroaryl.

The term "cycloheteroalkyl" as employed herein by itself or as part of another group refers to cycloalkyl where one or several carbon atoms are exchanged for a heteroatom.

The term "cycloalkenyl" as employed herein by itself or as part of another group refers to cycloalkyl containing one or several carbon-carbon double bonds, such as cyclopentenyl and cyclohexenyl.

The term "cycloheteroalkenyl" as employed herein by itself or as part of another group refers to cycloheteroalkyl where one or more bonds between carbons, carbon and heteroatom, or heteroatoms are double.

The term "aryl" as employed herein by itself or as part of another group refers to phenyl which may optionally be fused with 1 or 2 cycles which are independently selected of each other from the group consisting of cycloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, aryl and heteroaryl, and in which one or more hydrogens may be optionally substituted by halogen or alkyloxy.

The term "aryl" as employed herein by itself or as part of another group also refers to phenyl in which one or more hydrogens may be substituted by alkyl, fluorinated alkyl, alkenyl, fluorinated alkenyl, cykloalkyl, fluorinated cykloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, heteroaryl and/or a functional group, and which may be optionally fused with 1 or 2 cycles which are independently selected from each other from the group consisting of cycloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, aryl and/or heteroaryl.

The term "heteroaryl" as employed herein by itself or as part of another group refers to a 5- to 12-membered aromatic ring, preferably 5- to 6-membered aromatic ring, which includes one or more heteroatoms, which may be optionally fused with 1 or 2 cycles which are independently selected from each other from the group consisting of cycloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, aryl and heteroaryl.

The term "heteroaryl" as employed herein by itself or as part of another group also refers to a 5- to 12-membered

aromatic ring, preferably 5- or 6-membered aromatic ring, which includes one or more heteroatoms, and in which one or more hydrogens may be substituted by alkyl, fluorinated alkyl, alkenyl, fluorinated alkenyl, cykloalkyl, fluorinated cykloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, alkynyl, aryl, heteroaryl and/or a functional group, and which may be optionally fused with 1 or 2 cycles which are independently selected from each other from the group consisting of cycloalkyl, cycloheteroalkyl, cycloalkenyl, cycloheteroalkenyl, aryl and/or heteroaryl.

The term "halogen" as employed herein by itself or as part of another group refers to chlorine, bromine, fluorine and iodine with chlorine being preferred.

The term "heteroatom" as employed herein by itself or as part of another group refers to nitrogen, oxygen or sulphur, to which one or more hydrogens may be connected according to valence and in the case of nitrogen one oxygen may be optionally connected to it by donor-acceptor bond, thus forming N-oxide.

The term "heavy atom" refers to an atom whose mass is higher than 2 daltons.

The term "functional group" as employed herein by itself or as part of another group refers to amino, alkylamino, dialkylamino, aryloxy, alkoxy, arylamino, heteroarylamino, hydroxy, alkylhydroxy, fluorinated alkylhydroxy, cyano, carboxy, alkylcarboxy, carboxyalkyl, arylcarboxy, carboxyaryl, halogen, nitro, hydroxyamino, acyl, fluorinated acyl, nitroso, sulfonyl, sulfinyl, thio, alkylthio, arylthio, aminoguanidino, aminohydroxyguanidino, iminoguanidino, imino-hydroxyguanidino, guanidino, hydroxyguanidino,

guanidinoamino, hydroxyguanidinoamino, hydroxyguanidinoimino or guanidinoimino.

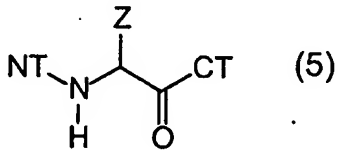
The term "fused" as employed herein by itself or as part of another group refers to two or three cycles having one or more common atoms, the preferred maximum number of fused cycles being three.

The term "substituted" refers to the fact that, in a term connected with it, one or more hydrogens are substituted by alkyl, fluorinated alkyl, alkenyl, fluorinated alkenyl, alkynyl, fluorinated alkynyl, cycloalkyl, fluorinated cycloalkyl, cycloheteroalkyl, fluorinated cycloheteroalkyl, cycloalkenyl, fluorinated cycloalkenyl, cycloheteroalkenyl, fluorinated cycloheteroalkenyl, aryl, fluorinated aryl, heteroaryl, fluorinated heteroaryl and/or a functional group. Moreover, if the structure connected with the term "substituted" is a cyclic structure fused with another cyclic structure or other cyclic structures then these latter cyclic structure(s) may also be substituted.

The term "fluorinated" as employed herein by itself or as part of another group refers to the fact that, in the following term, one or several hydrogens are substituted with fluorine.

The term "aminoacid" as employed herein by itself or as part of another group refers to alanine, arginine, asparagine, aspartic acid, p-benzoyl-phenylalanine, β-cyclohexyl-alanine, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, β-(2-naphthyl)-alanine, β-(1-naphthyl)-alanine, norleucine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, 3,4-dichlorophenylalanine, 4-fluorophenylalanine, 4-

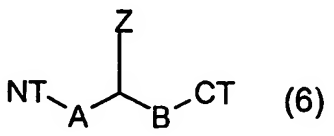
nitrophenylalanine, 2-thienylalanine, 3-benzothienylalanine, 4-cyanophenylalanine, 4-iodophenylalanine, 4-bromophenylalanine, 4,4'-biphenylalanine, pentafluorophenylalanine, β,β-diphenylalanine, in either D- or L-conformations, D-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, as well as other substances having the following general structure (5):



(5)

in which Z is H, X or $-CH_2X$ where X is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, or a functional group; NT is H, or a functional group or bond to another aminoacid; and CT is a functional group or bond to another aminoacid; the substance according to formula (5) being in either D- or L-conformation.

The term "aminoacid analogue" as employed herein by itself or as part of another group refers to a substance having the following general structure (6):



(6)

wherein A is nitrogen or carbon to which is attached hydrogen or methyl according to valence,

and wherein B is carbon to which is attached hydrogen or oxygen according to valence,
and wherein each possible asymmetric centre is in either R or S configuration,

and wherein Z is H, X or -CH_2X where X is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, or a functional group; NT is H, or a functional group, a bond to another aminoacid, or a bond to another aminoacid analogue; and CT is a functional group, a bond to another aminoacid, or a bond to another aminoacid analogue.

The term "ring atoms" is used herein to describe the atoms in the compound that form the actual ring. It will be understood in this regard that the absence of any of the ring atoms will result in the opening of the ring.

Some of the compounds of the invention bind to an MSH-receptor. By the term "bind to an MSH-receptor" is in this context intended a capacity of the compound of the invention to compete for the binding of [^{125}I]NDP-MSH at an MSH-receptor, the MSH-receptor preferably being selected from the group of the MC1, MC3, MC4 or MC5 receptor, with the MC4 receptor being most preferred, using a binding assay such as that described in Example 2 or Example 25. In a further meaning the term "to bind to

an MSH-receptor" is in this context intended that the K_i-value for the compound of the invention, determined using a method described in Example 2 or 25, is less than 1,000,000 nM, preferably less than 100,000 nM, more preferably less than 10,000 nM, somewhat more preferably less than 1,000 nM, even somewhat preferably less than 100 nM and most preferably less than 50 nM. Most preferably, the compound of the invention has a k_i of less than 1,000 nM or less than 50 nM for an MC4 receptor.

The term "Peptide residue" as used herein refers to a linear structure formed from amino acid residues and/or aminoacid analogue residues connected together with amide bonds, and/or

covalent bond between B of a residue complying to structure (6) and N of a residue complying to structure (5), and/or

covalent bond between the carbonyl carbon of a residue complying with structure (5) and A of a residue complying with structure (6), and/or

covalent bond between B of a residue complying with structure (6) and A of a residue complying with structure (6), the B and A not residing in the same residue,

the preferred maximum of the number obtained by calculating the sum of the number of amino acid residues and amino acid analogue residues being 4.

The term "amino acid residue" as used herein refers to a fragment of compound (5) in which H and/or CT are missing.

The term "amino acid analogue residue" as used herein refers to a fragment of compound (5) in which NT and/or CT are missing.

The invention also provides pharmaceutical compositions comprising a compound of the invention together with one or more adjuvants, carriers or excipients. Such compositions may be used for administration to an animal, mammal or to a human, for diagnosis, prevention or therapeutic treatment of diseases, in particular conditions involving MSH-receptors. Examples of such MSH-receptor related conditions that may be positively affected by administration of the compounds of the invention are fever, pain, chronic inflammatory diseases, memory disturbances in particular in elderly people, including Alzheimer's disease. Moreover positive effects may be obtained on the regeneration of nerves after nerve injuries, on psychomotor functions, in particular positive effects on pathological psychomotor functions of psychiatric conditions such as e.g. catatonic conditions. The compounds of the invention may also be used for mediating anti-epileptic, anti-inflammatory and anti-pyretic effects, and/or for modulating signaling functions in both the brain and the periphery. Another important use of the compounds of the invention may be the treatment of weight disorders (e.g. overweight and underweight), in particular when the weight disorder is related to an eating disorder, such as excessive food intake, reduced food intake, bulimia and/or anorexia, with respect to the latter in particular anorexia nervosa of humans.

A particularly important aspect of the invention is the use of the compounds of the invention for the treatment of eating disorders, in particular for the treatment of eating disorders related to underweight, cachexia or

anorexia of any cause in humans. In these conditions the administration of a compound of the invention may increase food intake, which may improve the patient's general condition, increase or restore their body weight and/or prolong their life. In particular the administration of the compound of the invention may be beneficial to elderly patients, senile patients, AIDS patients, cancer patients, and patients treated with cancer chemotherapeutics, as these patients often suffer from lack of appetite, which often leads to decreased food intake and severe weight loss. Yet another important embodiment of the invention is the administration of a compound of the invention to an animal to increase its rate of growth. In particular the latter is desired in animal breeding for meat production. A very specific embodiment of the present invention constitutes the intra peritoneal administration of Compound Q1 to rats for increasing food intake.

It is well known in the clinics that progressive inanition or wasting is a fundamental component of a complex phenomenon known as the anorexia/cachexia syndrome (ACS) of malignancy or AIDS. Weight loss can be seen in the full spectrum of patient care settings: as a presenting complaint, defining condition, treatment-related toxicity, or as a hallmark of impending death (Ottery et al., Semin. Oncol. 1998, 25, 35-44). In such cases the administration of a compound the invention may improve the patient's condition, and may in many cases even be life saving or at least increase the patient's life span, as well as quality of life.

Compounds of the invention may be used to induce penile erection. In particular they may be useful for inducing penile erection in relation to impotency of central or of psychological origin. However, they may also useful for

treatment of cases of impotency of peripheral origin as well as of any other origin of impotency.

Compounds of the invention may also be useful for the treatment of unwanted or prolonged penile erection, such as e.g. in priapism. Prolonged penile erection including that of priapism is generally an unwanted, not seldom painful, condition which may effectively be treated by compounds of the invention. Particularly useful for treatment of prolonged penile erection may be the MSH-receptor blocking compounds of the invention.

Other important uses of the compounds of the invention may be for the treatment of disturbances in: 1) placental development, 2) aldosterone synthesis and release, 3) thyroxin release, 4) spermatogenesis, 5) prolactin and FSH secretion, 6) sebum and/or pheromone secretion, 7) blood glucose levels, 8) natriuresis, and 9) intrauterine foetal growth. Moreover, compounds of the invention may be used for the treatment of uterine bleeding in women. Other important uses may constitute the control of blood pressure, heart rate, vascular tone and brain blood flow, blood glucose levels, events surrounding parturition, and/or to afford neuroprotection.

Compounds of the invention may also afford improvement in conditions associated with damages to neurons both in the central nervous system and in the periphery. Besides being neuroprotective this effect may be brought about by affording increased regeneration of the neural tissue and its associated elements.

The compounds of the invention may also be used in the treatment of conditions related to motivation, learning, memory, behaviour, inflammation, body temperature, pain perception, nerve growth and/or ovarian weight.

Some compounds of the invention may also be used for the treatment of disorders of muscle, in particular disorders of striated muscle. Particularly susceptible to treatment with a compound of the invention may be dystrophies of muscles, myositis, autoimmune diseases of muscle, infantile spinal atrophy, and/or hypotonia of muscle. Also conditions affecting heart muscle may be susceptible to treatment by compounds of the invention including dystrophies of heart muscle, inflammation of heart muscle, myositis in the heart, and/or autoimmune disorders of the heart.

The compounds of the invention may also be used for the treatment of spinal cord injuries.

Quite specific embodiments of the invention are directed to compounds which may decrease the formation of interleukin 1 (IL-1), interleukin 6 (IL-6), and/or tumour necrosis factor-α(TNF-α), to afford decreased production of nitric oxide and downregulate the activity of nitric oxide synthase (NOS). Other embodiments of the invention are directed to compounds which may stimulate the production of interleukin 8 (IL-8) and/or interleukin 10 (IL-10). Yet other embodiments of the invention are directed to compounds which may produce an effect opposite to that described in regard of IL-1, IL-6, TNF-α, nitric oxide, NOS, IL-8 and IL-10.

Compounds of the invention may also be used to treat inflammatory conditions. This includes inflammation to immunological reactions, unknown causes and inflammation caused by infections (e.g viral, bacterial, protozoan, helmintic, etc.) both in the periphery and in the central nervous system.

Examples of such conditions for which administration of the compound of the invention may induce beneficial effects include inflammation of any type and any origin. In particular inflammation or any related condition as well as any condition involving the action of macrophages, neutrophils, monocytes, keratinocytes, fibroblasts, melanocytes, pigment cells and endothelial cells. Moreover included are conditions caused by or associated with increased production and/or release of inflammatory cytokines such as interleukins, in particular interleukin 1 (IL-1), interleukin 6 (IL-6), and tumour necrosis factor-α (TNF-α). Included are also conditions associated with increased production of nitric oxide (NO) as well as upregulated activity of nitric oxide synthase (NOS). Moreover, some compounds of the invention may be useful for treating conditions related to the testis and ovary.

In the present specification "increased production" refers to increased formation, increased release, or increased amount of an endogenous compound locally, regionally or systemically in a patient compared to the amount of said endogenous compound in a healthy individual. In the present specification "upregulated" refers to an increased activity or amount of the compound compared with that in a healthy individual.

In the present specification "decreased production" refers to decreased formation, decreased release, or decreased amount of an endogenous compound in a patient compared to the amount of said endogenous compound in a healthy individual. In the present specification "downregulated" refers to a decreased activity or amount of the compound compared with that in a healthy individual.

In particular, positive treatment effects or preventive effects may be seen in conditions where inflammation or inflammatory like conditions are caused by or are associated with one or more of the following: allergy, hypersensitivity, bacterial infection, viral infection, inflammation caused by toxic agent, fever, autoimmune disease, radiation damage by any source including UV-radiation, X-ray radiation, γ-radiation, α- or β-particles, sun burns, elevated temperature, and mechanical injury. Moreover, inflammation due to hypoxia, which is optionally followed by reoxygenation of the hypoxic area, is typically followed by severe inflammation, which condition may be positively affected by treatment with a compound of the invention.

In very specific embodiments of the invention a compound of the invention may be administered for the prevention or therapeutic treatment of inflammatory diseases of the skin (including the dermis and epidermis) of any origin, including skin diseases having an inflammatory component. Specific examples of this embodiment of the invention include treatment of contact dermatitis of the skin, sunburns of the skin, burns of any cause, and inflammation of the skin caused by chemical agent, psoriasis, vasculitis, pyoderma gangrenosum, discoid lupus erythematosus, eczema, pustulosis palmo-plantaris, and phemphigus vulgaris.

Also within the scope of the invention is the administration of a compound of the invention for the treatment of an inflammatory disease in the abdomen, including an abdominal disease having an inflammatory component. Specific examples of treatment of such disease with a compound of the invention are gastritis, including gastritis of unknown origin, gastritis perniciosa (atrophic gastritis), ulcerous colitis (colitis

ulcerosa), morbus Crohn, systemic sclerosis, ulcus duodeni, coeliac disease, oesophagitis and ulcus ventriculi.

The invention also relates to the administration of a compound of the invention for treatment of systemic or general and/or local immunological diseases, including those of an autoimmune nature, and other inflammatory diseases of a general nature. Specific examples include treatment of rheumatoid arthritis, psoriatic arthritis, systemic sclerosis, polymyalgia rheumatica, Wegener's granulomatosis, sarcoidosis, eosinophilic fasceitis, reactive arthritis, Bechterew's disease, systemic lupus erythematosus, arteritis temporalis, Behcet's disease, morbus Burger, Good Pastures' syndrome, eosinophilic granuloma, fibromyalgia, myositis, and mixed connective tissue disease. Included therein is also arthritis, including arthritis of unknown origin.

Further included in the invention is administration of a compound of the invention for treatment of a disease of the peripheral and/or central nervous system related to inflammation. Included in this aspect of the invention is the treatment of cerebral vasculitis, multiple sclerosis, autoimmune ophthalmitis, and polyneuropathia. Comprised by the invention is also the administration of a compound of the invention for treatment of an inflammation of the central nervous system to prevent apoptotic cell death. Moreover, as some compounds of the invention may show a distinct ability to induce nerve regeneration, positive treatment effects may often be seen in central nervous system diseases involving damage of cells in this region. This aspect of the invention also includes treatment of traumatic injuries to the central nervous system, brain edema, multiple sclerosis, Alzheimer's disease, bacterial

and viral infections in the central nervous system, stroke, and haemorrhagia in the central nervous system.

Comprised by the invention is also the administration of a compound of the invention for treatment of diseases of the eye and tear glands. Part of this aspect of the invention refers to, but is not limited to, diseases of eye and tear glands related to inflammation. Specific examples of such diseases comprise anterior and posterior uveitis, retinal vasculitis, opticus neuritis, Wegener's granulomatosis, Sjögren's syndrome, episcleritis, scleritis, sarcoidosis affecting the eye, and polychondritis affecting the eye.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases of the ear related to inflammation, specific examples of which include polychondritis affecting the ear and external otitis.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases of the nose related to inflammation, specific examples of which are sarcoidosis, polychondritis and mid-line granuloma of the nose.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases related to inflammation of the mouth, pharynx and saliva glands. Specific examples include Wegener's granulomatosis, mid-line granuloma, Sjögren's syndrome and polychondritis in these areas.

Included in the invention is also the administration of a compound of the invention for the treatment of diseases related to inflammation in the lung. Specific examples

include treatment of idiopathic alveolitis, primary pulmonary hypertension, bronchitis, chronic bronchitis, sarcoidosis, alveolitis in inflammatory systemic disease, pulmonary hypertension in inflammatory systemic disease, Wegener's granulomatosis and Good Pastures' syndrome.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases related to the inflammation of the heart. Specific examples include treatment of pericarditis, idiopathic pericarditis, myocarditis, Takayasus' arteritis, Kawasaki's disease, coronary artery vasculitis, pericarditis in inflammatory systemic disease, myocarditis in inflammatory systemic disease, endocarditis and endocarditis in inflammatory systemic disease.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases related to inflammation of the liver. Specific examples include treatment of hepatitis, chronic active hepatitis, biliary cirrhosis, hepatic damage by toxic agent, interferon induced hepatitis, hepatitis induced by viral infection, liver damage induced by anoxia and liver damage caused by mechanical trauma.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases related to inflammation of the pancreas. Specific examples include treatment (and prevention) of diabetes mellitus, acute pancreatitis, chronic pancreatitis.

Within the scope of the invention is also the administration of a compound of the invention for the

treatment of diseases related to the inflammation of the thyroid. Specific examples of these embodiments of the invention include treatment of thyreoiditis, autoimmune thyreoiditis, and Hashimoto's thyreoiditis.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases related to inflammation of the kidney. Specific examples include treatment of glomerulonephritis, glomerulonephritis in systemic lupus erythematosus, periarteritis nodosa, Wegener's granulomatosis, Good-Pastures' syndrome, HLAb27 associated diseases, IgA nephritis (IgA = Immunoglobulin A), pyelonephritis, chronic pyelonephritis and interstitial nephritis.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases related to the inflammation of the joints. Specific examples include treatment of Bechterew's disease, psoriatic arthritis, rheumatoid arthritis, arthritis in colitis ulcerosa, arthritis in morbus Crohn, affection of joints in systemic lupus erythematosus, systemic sclerosis, mixed connective tissue disease, reactive arthritis, and Reiter's syndrome. Moreover, included in this embodiment of the invention is the treatment of arthrosis of any joint, in particular arthrosis of finger joints, the knee and the hip.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of diseases related to the inflammation of blood vessels. Specific examples include treatment of arteritis temporalis, periarteritis nodosa, arteriosclerosis, Takayasus' arteritis and Kawasaki's

disease. Particularly advantageous may be the capacity of a compound of the invention to afford protection against and prevention of arteriosclerosis. This may in part due to the capacity of a compound of the invention to prevent the induction of inducible nitric oxide synthase (iNOS) caused by the action of oxidized Low Density Lipoprotein on endothelial cells and blood vessel walls.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of drug induced disorders of the blood and lymphoid system, including the treatment of drug induced hypersensitivity (including drug hypersensitivity) affecting blood cells and blood cell forming organs (e.g. bone marrow and lymphoid tissue). Specific embodiments of this aspect of the invention include the treatment of anemia, granulocytopenia, thrombocytopenia, leukopenia, aplastic anemia, autoimmune hemolytic anemia, autoimmune thrombocytopenia, autoimmune granulocytopenia.

A compound of the invention may also be administered for the treatment of fast allergic disorders (Type I allergy). Included in this embodiment of the invention is the treatment of anaphylactic reactions, anaphylactoid reactions, asthma, asthma of allergic type, asthma of unknown origin, rhinitis, hay fever, and pollen allergy.

Within the scope of the invention is also the administration of a compound of the invention for the treatment of inflammation related to infections of any origin. Specific examples include the treatment of inflammation secondary to infections caused by virus, bacteria, helminths and/or protozoae.

Within the scope of the invention is also the administration of a compound of the invention for the

treatment of inflammation related to trauma and tissue injury of any origin.

Compounds of the invention may be used to stimulate pigment formation in epidermal cells. Accordingly, compounds of the invention may also be useful for inducing skin tanning for cosmetic reasons, for treatment of vitiligo, or any other condition where darkening of skin colour is desired. Compounds of the invention may also be used to inhibit pigment formation in cells of the skin, and hence they may be useful for inducing lighter skin colour for cosmetic reasons, or during any condition where a lighter colour of skin is desired.

Compounds of the invention may also be used in the treatment, including preventive treatment, of drug addiction. Such treatments include, but are not limited to the treatment of addiction related to morphine, cocaine, amphetamine, alcohol and/or other narcotics, treatment of withdrawal symptoms as well as the elimination/reduction of reward effects caused by drugs.

Compounds of the invention may also be used for inhibiting the formation of the second messenger element cyclic adenosine 3',5'-monophosphate (cAMP). In particular, such formation of cAMP is desired for eliciting the specific pharmacological effects of the compounds of the invention when administered to a living organism, in particular a human. However, the inhibition of cAMP formation may also be of great value in cells or crushed cell systems *in vitro*, e.g. for analytical or diagnostic purposes.

Compounds of the invention may also be used for inducing formation of the second messenger element cAMP. In particular, such formation of cyclic adenosine 3',5'-

monophosphate (cAMP) is desired for eliciting the specific pharmacological effects of some compounds of the invention when administered to a living organism, in particular a human. However, the induction of cAMP formation may also be of great value in cells or crushed cell systems *in vitro*, e.g. for analytical or diagnostic purposes.

The compounds of the invention may be manufactured using any convention chemical technique.

Compounds of the present invention may be used in radioactive form, including having radioactive labels. Such a radioactively labelled compound of the invention may particularly be useful for analytical and/or diagnostic purposes. Compounds of the invention may be manufactured so as to incorporate radioactive iodine or tritium, or any other suitable radionuclide. Such a radioactively-labeled compound may be used in radioligand binding for the quantification of specific melanocortin receptors, for the analysis of dissociation constants (K_is or K_ds) of drugs competing with specific subtypes of melanocortin receptors, and/or for the localization of MC-receptors in tissues and tissue sections e.g. by the use of receptor autoradiographic techniques. Principles of radioligand binding and receptor autoradiography are well known in the art. As an alternative, the compound may be labeled with any other type of label that allows detection of the substance, e.g. a fluorescent label or biotin, and the resulting compound may be used for the similar purpose as the radioactively labeled compound.

Compounds of the invention may also be manufactured so as to incorporate a group that can be activated by light, in particular UV-light, the purpose of such activation being to obtain a compound useful for covalent labeling of

MC-receptors by use of the photoaffinity labeling technique. Photoaffinity labeling is a technique well known in the art which in the present context is useful for elucidating the structure and/or topological organisation of the MC-receptors. Thus photoactive derivatives of the compounds of the invention are also part of the present invention. Moreover, preferably photoactive derivatives of the compounds of the invention may also be made to incorporate an easily detectable group or label, such as e.g. a radioactive atom, a fluorescent group and/or biotin. (For further details in regard of photoaffinity labeling see: Leeb-Lundberg et al., J. Biol. Chem. 1984, 259, 2579 and Scimonelli & Eberle, FEBS Lett., 1987, 226, 134.)

Compounds of the invention may be labeled with gamma and/or positron emitting isotope(s). Such labeled compounds constitute very specific embodiments of the invention and may be administered systematically, or locally, to an animal, preferably a human. These labeled compounds are useful for imaging the *in vivo* levels and/or localization of MC-receptors by the use of well known techniques among which may be mentioned Scintigraphy, Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT). Using such methods, information on the distribution and/or quantities of the specific MSH-receptors in tissues of the animal or human subject to the investigation may be obtained, and such information may be of value for diagnosis of disease, in particular functional disturbances in the brain related to MSH-receptors.

Agonist and antagonist activities, as well as inverse agonistic actions of the compounds of the invention may be evaluated by various methods known in the art. Examples of such methods are measurement of second

messenger responses, in particular cAMP, the use of modified cell systems yielding colour reaction upon accumulation of second messenger elements such as cAMP, e.g. as described in Examples 3 and 26 or using Cytosensor Microphysiometer techniques (see Boyfield et al., Microphysiometer. Biochem Soc Trans. 1996, 24, 57S). In these tests tissues, native cells, cancer cells, immortalized cells, melanoma cells, astrocytes (see Zohar and Salomon, Brain Res. 1992, 576, 49-58), genetically engineered cells, or cells expressing MC-receptors from cloned genes (see e.g. Schiöth et al., Br. J. Pharmacol. 1998, 124, 75-82), may be used. Other methods useful for similar purposes constitute the administration of a compound of the invention to brain slices (either alone or in combination with natural or synthetic MSH-peptides, or MSH-receptor agonists) and the effect assessed by measurement of cAMP in the slices (Lezcano et al., Peptides. 1993, 14, 53-57). The activity of a compound of the invention may also in a similar way be assessed by measuring lipolysis, cAMP or adenylate cyclase activity in adipocytes after the administration of a compound of the invention alone or in combination with MSH-peptides, or MSH-receptor agonists (for an outline of the approach see Rudman, J. Pharmacol. Exp. Ther. 1975, 195, 532-539).

The effects of a compound of the invention may also be evaluated *in vitro* using organ bath techniques or *in vivo* in experimental animals. An effect of a compound of the invention may be observed after the administration of the compound alone or after administration in combination with natural or synthetic MSH-peptides, or MSH-receptor agonists.

The binding affinity for an MC-receptor of a compound of the invention may be assessed by using radioligand binding. A specific embodiment of this aspect of the

invention is given in Example 2. The binding affinity of a compound of the invention may also be assessed by using autoradiography, e.g. to assess the binding affinity to an MC receptor in the central nervous system (Lindblom et al., Brain Res., 1998, 810, 161-171). The affinity of a compound of the invention may also be assessed using receptors expressed in cell lines by using methods well known in the art (Schiöth et al., Eur. J. Pharmacol., Mol. Pharm. Sect. 1995, 288, 311-317; Schiöth et al. Pharmacol. Toxicol. 1996, 79, 161-165).

Orexigenic and anorexigenic effects of a compound of the invention may be tested by administering the compound to an animal and studying the amount of food intake per time unit of the animal. The long and short term effects of the compound of the invention on body weight may also be studied using methods well known in the art (see e.g. Kask et al., Biochem. Biophys. Res. Commun. 1998, 245, 90-93; Kask et al. Biochem. Biophys. Res. Commun. 1998, 248, 245-249; Skuladottir et al., Long term orexigenic effects of a novel selective MC4 receptor antagonist, Brit. J. Pharmacol. (in press). The treatment effects of the compounds of the invention in anorexia may be assessed by the administration of a compound of the invention to animals serving as models for anorexia, such as e.g. to animals suffering from anorexia due to zinc deficiency (Essatara et al., Physiol. Behav. 1984, 32, 469-474), or to animals suffering from anorexia due to immobilisation induced stress (Ferrari et al., Eur. J. Pharmacol. 1992, 210, 17-22), or to animals suffering from anorexia due to genetic faults, such as e.g. that found in anx/anx mice (Broberger et al., J. Comp. Neurol. 1997, 387, 124-135), or to anorexia in the case of food-restricted hyperactivity (see Altemus et al., Pharmacol. Biochem. Behav., 1996, 53, 123-131), and studying parameters such as change of body weight, change of fat

depots, changes in muscle mass, condition of animal, and other biological, physiological or biochemical parameters.

In a similar way the treatment effects of a compound of the invention in obesity may be assessed by using one of several obesity models well known in the art. Such models comprise (but are not limited to) the administration of the compound of the invention to obese Zucker rats (Kasiske et al., Hypertension. 1992, 19, 110-115) or to animals made obese by feeding of a highly palatable diet (Wilding et al., J. Endocrinol. 1992, 132, 299-304), and studying parameters such as change of body weight, change of fat depots, changes in muscle mass, condition of animal, and other biological, physiological or biochemical parameters.

The assessment of the effectiveness of a compound of the invention to affect parameters related to feeding and bodyweight, e.g. by using methods similar to those mentioned in the two preceding paragraphs, constitute important tools in the selection of the most clinically useful of the compounds of the invention and therefore also forms part of the invention.

Compounds of the present invention may be covalently or non-covalently bound to one or several of other optional molecule(s) of any desired structure(s); the thus formed modified compound or complex being useful for the same purposes as described above for the compounds of the invention, as well as is disclosed further below.

Compounds of the invention may be useful for the treatment and diagnosis of disorders in animals, in particular a mammal, which most preferably is a human.

In some preferred embodiments of the invention a rapid breakdown of the compound of the invention by endogenous enzymatic system(s) is desired as this will lead to a drug with rapid action and short half life. However, it is recognized that the minor alterations of the compound of invention such as e.g. addition of N-methyl groups, particularly to a nitrogen-atom of the peptide backbone of a compound of the invention, may lead to compounds that are less susceptible to enzymatic breakdown and thus increased half life in the body. Such minor alterations may also lead to compounds with increased ability to penetrate biological membranes such as the blood-brain barrier, or leading to compounds that are better absorbed from the gastro-intestinal tract. Example of an N-methyl substituted compound of the invention is Compound Q16.

The capacity of a compound of the invention to afford MSH-receptor desensitization and/or MSH-receptor down regulation is in some embodiments of the invention a very desired action caused by the compound of the invention. Moreover, in further embodiments of the invention upregulation and/or increased expression of an MSH-receptor may be afforded by the administration of the compound of the invention, which is also be a highly desirable action caused by the compound of the invention.

A compound of the invention may be used in the form of a pro-drug. By pro-drug is in this context intended a chemical compound from which the compound of the invention is formed in the body upon the administration of said pro-drug. Pro-drugs include, but are not limited to, esters of a compound of the invention, such as acetate, benzoate, pivaloate, etc.. In particular embodiments of the invention the administration of the compound in the form of a pro-drug is considered particularly advantageous such as e.g. for improving

uptake from the gastro-intestinal tract, passage through the blood-brain barrier and prevention of a too rapid degradation in the body.

Compounds of the present invention, and their pro-drugs, may be used in the form of pharmaceutically-acceptable acid addition salts derived from inorganic or organic acids. These salts include, but are not limited to, the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, sulfate, bisulfate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxy-ethanesulfonate, lactate, maleate, methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, trifluoroacetate, tosylate, and undecanoate, with trifluoroacetate being preferred. These salts can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the free base with a suitable organic or inorganic acid.

Compounds of the invention, or their pro-drugs, may be administered in therapeutically effective amounts. By a "therapeutically-effective amount" is meant a sufficient amount of the compound to treat or prevent disorders. The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder or the protective effect sought; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, gender and diet of the patient; the time of administration,

route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; and/or drugs used in combination or coincidental with the specific compound employed.

The total daily dose of a compound according to the invention administered in single or divided doses to person may be, for example, from about 0.001 to about 100 mg/kg body weight, or more usually, from about 0.1 to about 50 mg/kg body weight. Single dose compositions may contain such amounts or submultiples thereof to make up the daily dose. In general, treatment regimens according to the present invention comprise administering to a patient in need of such treatment from about 20 mg to about 2000 mg of the compound(s) of this invention per day in multiple doses or in a single dose. However, in severe cases and/or for acute treatment higher doses, such as up to 10,000 mg of one or several compounds of the invention may be administered in a single dose which may be given in multiple consecutively administered portions. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated, the particular treatment and the particular mode of administration.

Other dosage requirements may be required than stated in the fore-going paragraph depending on different dosage schedules, such as e.g. if the compound of the invention is given topically.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically-acceptable carriers, adjuvants, and vehicles as desired.

The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques. Liquid dosage forms for oral administration may include pharmaceutically-acceptable emulsions, microemulsions, solutions and suspensions containing inert diluents such as water. Such compositions may also comprise adjuvants, such as wetting agents; emulsifying and suspending agents; and sweetening, flavouring and perfuming agents. Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils are conventionally employed as a solvent or suspending medium. Fixed oils and fatty acids, such as oleic acid may be employed in the preparation of injectables.

The injectable formulation may be sterilized, for example, by filtration through a bacteria- or virus-retaining filter, by radiation, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use. There are various methods for delaying absorption of a drug known in the art such as, for instance, to administer the drug as a solution or suspension in oil. Injectable depot forms can also be made by forming microcapsule matrices of drugs and biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer and the

composition of the polymer, the rate of drug release can be controlled. Examples of other biodegradable polymers include polyorthoesters and polyanhydrides. The depot injectables can also be made by entrapping the drug in liposomes or microemulsions that are compatible with body tissues.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with suitable non-irritating excipients known in the art and having a melting point appropriate for such administration, that is of about 30°C. Solid dosage forms for oral administration may include capsules, tablets, pills, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings, for instance coatings which release the drug in the small intestine but not in the stomach. In regard of the preparation of tablets for oral administration particular reference is made to Pharmaceutical Dosage Forms, Vol. 1-3, Lieberman A et al., Eds., 2nd Ed. Marcel Dekker, New York 1989-90.

Compounds of the invention may also be administered topically, transdermally or by inhalation in the form of ointments, pastes, creams, lotions, gels, powders, solutions, sprays, patches or inhalants. The compound may be admixed under sterile conditions with a pharmaceutically-acceptable carrier and any preservatives or buffers that may be required. Ophthalmic formulations

are also contemplated as being within the scope of this invention.

The compound of the invention may also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic physiologically acceptable and metabolizable lipid capable of forming liposomes may be used. The present compositions in liposome form may contain, in addition to the compounds of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Vol. XIV, Academic Press, New York, N.Y. 1976, pp. 33 et seq..

Compounds of the invention may be administered in formulations that slowly release the compound thus allowing a sustained delivery of said compound over a prolonged period of time.

Compounds of the invention may be delivered to the preferred site in the body, such as e.g. to the brain, by using a suitable drug delivery system. Drug delivery systems are well known in the art. For example, compounds of the invention may be coupled to a carrier molecule making them lipophilic (see e.g. Toth, I., J. Drug. Targeting, 1994, 2, 217-239; Patel et al., Bioconjugate Chem., 1997, 8, 434-441). Other technologies that may be used to deliver the compounds of the invention to the desired sites in the body are vector mediated carrier systems (see e.g. Pardridge, WM, Pharmacol. Toxicol.

1992, 71, 3-10; Saito, Y. et al., Proc. Natl. Acad. Sci. USA 1995, 92, 10227-10231; Wu, D. and Pardridge, WM J. Pharmacol. Exp. Ther. 1996, 279, 77-83). Yet other examples of drug delivery technologies useful for the compounds of the present invention are the conjugation of the compound of the invention to an active molecule capable of being transported through a biological barrier (see e.g. Zlokovic, BV., Pharmaceutical Research 1005, 12, 1395-1406). A specific example constitutes the coupling of the compound of the invention to fragments of insulin to achieve transport across the blood brain barrier (Fukuta, M et al. Pharmaceutical Res. 1994, 11, 1681-1688). For general reviews of technologies for drug delivery suitable for the compounds of the invention see Zlokovic, BV, Pharmaceutical Res. 1995, 12, 1395-1406 and Pardridge, WM, Pharmacol. Toxicol. 1992, 71, 3-10.

The compounds of this invention may be administered alone or in combination with other agents.

Compounds of the invention may be administered together with peptidase and protease inhibitors to prevent or delay the breakdown of the compound of the invention and thereby prolong the duration of its pharmacological action in the body as well as its stability in the gastrointestinal tract when administered per orally. Peptidase/protease inhibitors that may be administered together with a compound of the invention may be selected, but are not limited in its selection, from the group of angiotensin converting enzyme inhibitors (ACE-inhibitors) such as e.g. Captopril (D-3-mercaptomethyl-propionyl-L-proline), Enaplapril, phosphoramidone, and amastatin.

The 3D-structure of a compound of the invention may be determined using computer molecular modelling, NMR

(Nuclear Magnetic Resonance) or X-ray crystallographic techniques. The 3D structure of the compound of the invention may be used as a template for the design of novel drugs for the control of eating behaviour or other aspects of the use of a compound of the invention as is disclosed herein.

The concentration of a compound of the invention or its prodrug in body fluids (e.g. plasma, serum), tissues, or in solution outside of the body, may be analyzed using any conventional technique such as HPLC, mass-spectrometry, radio-immunoassay, ELISA, light-spectrometry and NMR. Such analysis is particularly valuable in the assessment of the effectiveness of a treatment as it is desired that the concentration is kept within a therapeutic interval. The ability of a compound of the invention to be taken up via the gastrointestinal tract can be assessed by administering the compound per-orally and measuring the concentration of the compound of the invention in the blood plasma at timed intervals. The resistance of the compound of the invention to be subjected to first passage metabolism in the liver can be assessed either by administering it per-orally or intra-peritoneally and measuring the amount of drug entering into the blood circulation and, particularly in the case of per oral administration, accounting for the amount of compound not taken up over the gastrointestinal mucosa. The ability of Compound Q1 to give systemic effects after intra peritoneal administration (Example 5) indicates that the first passage metabolism (i.e. liver metabolism) does not take place completely. The assessment of pharmacokinetics, first passage metabolism, and ability of a compound of the invention to be absorbed via the gastrointestinal tract constitute important tools in the selection of the most clinically useful of the compounds

of the invention, and are therefore part of the invention.

Some of the compounds of the invention are capable of passing through the blood-brain barrier. The capacity of a compound of the invention to pass through the blood brain barrier may be assessed by measuring the concentration of the compound of the invention in blood, blood plasma or blood serum and comparing with the concentration that can be measured in the brain or cerebrospinal fluid. The capacity of a compound of the invention to pass through the blood brain barrier may also be assessed by observing the central nervous system pharmacological effects induced by the compound of the invention after its general administration to the animal. The assessment of the capacity of a compound of the invention to pass through the blood-brain barrier constitute important tools in the selection of the most clinically useful of the compounds of the invention, and is therefore part of the invention.

The compounds of the invention may also be used in treatment of intoxications brought about by ingestion of MSH-peptides.

The invention will now be described in greater detail by reference to a number of Examples which however are only given for purposes of illustration and must not be considered to limit the invention in any way.

DEFINITIONS

In the present context the term MC-receptor is *mutatis mutandis* having the same meaning as MSH-receptor.

In the present context "D-" denotes R configuration of α-aminoacid.

In the present context "L-" denotes S configuration of α-aminoacid.

ABBREVIATIONS

A number of abbreviations are used herein. These abbreviations are defined as follows:

α -MSH	α -melanocyte stimulating hormone (α-melanocortine)
Ac	acetyl
Ac_2O	acetic anhydride
Al	allyl
Ala	alanine
Aloc	allyloxycarbonyl
Aoa	8-aminooctanoic acid
Arg	arginine
Asp	aspartic acid
Aua	11-aminoundecanoic acid
Boc	tert.butyloxycarbonyl
Cys	cysteine

DiClPhe	3,4-dichlorophenylalanine
DIEA	N,N-diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	dimethylsulphoxide
DPPA	diphenylphosphorylazide
Fmoc	9-fluorenylmethoxycarbonyl
Fmoc-PAL-PEG-PS	5-(4-Fmoc-aminomethyl-3,5-dimethoxy)valeric acid attached to polyethylene-graft polystyrene support
Gly	glycine
HATU	7-azabenzotriazol-1-yl-oxy-1,1,3,3-tetramethyluronium hexafluorophosphate
His	histidine
HOAt	1-hydroxy-7-azabenzotriazole
HPLC	High Performance Liquid Chromatography
Lys	lysine
MeCN	acetonitrile
Nal	3-(2-naphthyl)alanine
Nal(1)	3-(1-naphthyl)alanine

NMePhe	N-methylphenylalanine
NMM	N-methylmorpholine
Orn	ornithine
Pbf	2,2,4,6,7 - pentamethyldihydrobenzofuran-5-sulfonyl
Pen	penicillamine
Pfp	pentafluorophenyl
Phe	phenylalanine
PyAOP	7-azabenzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
TBTU	7-benzotriazol-1-yl-oxy-1,1,3,3-tetramethyluronium tetrafluoroborate
TFA	trifluoroacetic acid
TIS	triisopropylsilane
Trp	tryptophan
Trt	trityl
SEM	Standard Error of the Mean

Legends to Figures

Fig. 1 Competition of Compound Q1 with [^{125}I][Nle4,D-Phe7] α-MSH binding for MSH-receptors in B16 melanoma cells.

Fig. 2 Influence of α-MSH and Compound Q1, and combinations thereof, on cAMP in B16 melanoma cells.

Fig. 3 2D structure of Compound Q1 with indices assigned to hydrogen atoms.

Fig. 4 NMR determined 3D structure of Compound Q1. Hydrogen atoms (except for one hydrogen atom forming a hydrogen bond), acetyl and amido terminals are not shown.

Fig. 5 Effect of Compound Q1 on food intake after intra peritoneal (i.p) injection to rats (220-270g). The graph shows the cumulated food intake at 2 and 4 hours after the i.p. injections of, respectively, saline (vehicle), 0.1 mg/kg Compound Q1 or 0.5 mg/kg Compound Q1. The number of rats tested are 5 to 8 as indicated. (Repeated measures ANOVA treatment effect $F(1,17)=2,34$ $P=0.12$, NS treatmentXtime interaction $F(2,17)=3.93$ $P<0.05$). *Indicates a significant difference at $p<0.05$.

Figs. 6 and 7 Ki values of compounds of the invention when binding to human MC1, MC3, MC4 and MC5 receptors as determined by competition with [^{125}I][Nle4,D-Phe7]α-MSH.

Example 1

Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, D-Nal^7, L-Cys-NH$_2$^{10}) α -MSH$_{5-10}$ trifluoroacetate (Compound Q1, SEQ ID NO: 5).

The peptide sequence was assembled on a solid support using "Pioneer" peptide synthesis system. Fmoc-PAL-PEG-PS (250 mg, 0.05 mmole) was placed into the peptide synthesis column. Then the Fmoc group was removed by 20% piperidine in DMF (5 min), support washed with DMF. Fmoc-Cys(Trt)-OPfp(150 mg, 0.2 mmole) and HOAt(27 mg, 0.2 mmole) were dissolved in 4 ml DMF and circulated through the column for 30 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-Trp(Boc)-OH (105 mg, 0.2 mmole), HATU (76 mg, 0.2 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-Arg(Pbf)-OH (130 mg, 0.2 mmole), HATU (76 mg, 0.2 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-D-Nal-OH (79 mg, 0.2 mmole), HATU (76 mg, 0.2 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-Gly-OH (59 mg, 0.2 mmole), HATU (76 mg, 0.2 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and

circulated through the column for 60 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-Cys(Trt)-OPfp(150 mg, 0.2 mmole) and HOAt(27 mg, 0.2 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Then the support was treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, then methanol, then dichloromethane and dried *in vacuo*. The resin was treated with 5 ml of deprotection mixture (TFA - water - 1,2-ethanedithiol - TIS, 92.5:2.5:2.5:2.5) and allowed to stand at room temperature for 3 hours. It was filtered, washed on the filter with TFA, the united filtrate was concentrated *in vacuo* at room temperature. Dry ether was added, the precipitate formed was filtered off and washed on the filter with ether, then dried *in vacuo* over KOH. The product was dissolved in 3 ml DMSO and placed under argon into a thermostat at 65°C for 36 hours. Then the solvent was evaporated at room temperature *in vacuo*. The residue was dissolved in 1 ml of 60% MeCN in water, solution divided into three portions and placed into centrifuge tubes, each of them was diluted with 0.1 % aqueous TFA to 1.5 ml volume. It was centrifuged and the clear solutions were used for a semi-preparative HPLC (10 x 250 mm column, Vydac RP C18, 90A, 201HS1010, eluate - 17 % MeCN in water + 0.1% TFA, detection at 230 nm. Fractions, containing the main peak, were pooled and lyophilized. A white powder formed. Yield 11.2 mg(23 %). R_f 0.70(1-butanol - AcOH - water, 4:1:1, Merck Silica Gel 60 F_{254} plates). k' 2.75(17% MeCN in 0.1% TFA). Plasma desorption mass-spectrometry: 861.1(M + H)

Example 2

Demonstration of the capacity of Compound Q1 to bind to melanocortin (MSH) receptors in mouse B16 melanoma cells

Cell culture.

B16 mouse melanoma cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, 1% MEM non-essential amino acid and 1% MEM vitamin solution, 100 IU penicillin/ml and 100 microgram streptomycin/ml at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Cells grown in monolayers were detached from the culture flasks and collected by low speed centrifugation (700*g*).

Receptor binding studies

MSH-receptor-binding was done essentially as described (Xia et al., Cancer Letters, 1996, 98, 157-162), in principle according to earlier described methods (Eberle et al., J. Recept. Res. 1991, 11, 311-322). In brief the collected cells were washed, distributed into 96 well plates and sedimented onto the well bottoms by centrifugation. The cells were then incubated for 2 h at 37°C, with 0.1 ml binding buffer in each well containing [^{125}I][Nle^4, D-Phe^7]α-MSH (0.2 nM), different concentrations of the Compound Q1 peptide in different wells at 37°C in MEM medium with Eagle's salts, 25 mM HEPES, pH 7.4, 0.2% bovine serum albumin, 1 mM 1,10-phenanthroline, 0.5 microgram leupeptin/ml and 200 microgram bacitracin/ml. After incubation the plates were put on ice, centrifuged and the cells washed with 0.2 ml of ice-cold binding buffer, centrifuged and the binding buffer was sucked off. The finally sedimented and washed cells were then detached from the plates with 0.1 ml of 0.1 N NaOH. Radioactivity was counted by using a Wallac, Wizard automatic gamma counter. The competition data

were analysed by law of mass-action computer modelling essentially as described (Bergström & Wikberg, Acta Pharmacol. Toxicol. 1986, 59, 270-278).

Results

As is seen from Fig. 1 increasing concentration of the Compound Q1 peptide caused a dose dependent inhibition of the binding of [^{125}I]-NDP-MSH to the B16 melanoma cells. By fitting the data to equations derived from the law of mass-action the dissociation constant (K_i) of Compound Q1 for the mouse B16 melanoma cell MSH receptor was found to be 2.51± 0.22 micromolar (mean ± SEM; n=3)

Example 3

Demonstration of the antagonistic capacity of Compound Q1 using mouse melanoma cells

Cell culture.

B16 mouse melanoma cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, 1% MEM non-essential amino acid and 1% MEM vitamin solution, 100 IU penicillin/ml and 100 microgram streptomycin/ml at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Cells grown in mono-layers were detached from the culture flasks and collected by low speed centrifugation (700*g*).

Stimulation of cell cAMP

For cAMP measurements the cells were detached from 60-80% confluent adherent cultures using Hank's balanced salts containing 0.5 mM EDTA and incubated for 30-60 min at 37°C in ordinary growth medium containing 0.5 mM of the phosphodiesterase inhibitor 3-*iso*-butyl-1-methyl-xanthine (IBMX). 20 µl aliquots of appropriate dilutions of test compounds in growth medium were prepared in 96 well

microtitre plates and placed in a water bath at 37°C. For the stimulation about $1.5x10^5$ cells in 180 µl were quickly added to each well to obtain immediate mixing. After 20 min 20 µl of 4.4 M perchloric acid were added, mixed, neutralized after a few minutes by addition of 20 µl base (5 M KOH, 1 M Tris) and centrifuged.

Determination of cAMP concentrations

20 µl of acid treated supernatant obtained above were mixed with 50 µl buffer (100 mM Tris-Cl, 250 mM NaCl, 10 mM EDTA, 0.1% mercaptoethanol, 0.5 mM IBMX, pH=7.4) containing 0.01 µCi [^{3}H]cAMP (Amersham, 1.04 TBq/mmol, 1 µCi/µl, product no.: TRK304). 200 µl of the same buffer containing a 1:16 diluted porcine adrenal gland bark extract (prepared as described by Nordstedt and Fredholm, Anal. Biochem., 1990, 189, 8258-8262) were added and the microtitre plates were incubated for at least 2 hours at 4°C. A standard curve was prepared in the same manner with dilutions of cAMP covering the range 2 µM - 0.5 nM.

After completion of incubation the solutions were filtered over GF-B glassfibre filters (Whatman) and washed briefly with ca. 2 ml ice-cold washing buffer (50 mM Tris-Cl, pH=7.4). Radioactivity on the filters was measured after addition of scintillation liquid. Stimulation experiments were determined in quadruplicate and standard curves in duplicate.

Results

The results are shown in Fig. 2. As can be seen from the Figure α-MSH 10^{-13} - 10^{-6} M caused an 8-fold increase in cAMP of the B16 melanoma cells. However, in the presence of 10 nM or 100 nM of Compound Q1 the α-MSH response on cAMP became practically abolished. As an be seen from the Figure Compound Q1 alone, in concentrations 10^{-13} to 10^{-4}

M, did not cause any effect on cAMP in the B16 melanoma cells.

Example 4

Determination of the 3D-structure of Compound Q1 using NMR

Methods

The determination of the 3D-structure of Compound Q1 in water solution at 4°C and pH 4.6 using NMR experiments was carried out at 600 MHz ^{1}H-frequency on a Bruker DMX 600 NMR spectrometer at 4°C. The ^{1}H resonance assignment of Compound Q1 was based on two-dimensional clean TOCSY (Griesinger et al., J. Amer. Chem. Soc. 1988, 110, 7870-7872) and ROESY (Bax and Davis, J. Magn. Reson. 1985, 63, 207-213) spectra. The clean TOCSY spectra were recorded in H_2O solution with 70 ms mixing time using the mixing scheme of Briand and Ernst (Chem. Phys. Lett. 1991, 185, 276-285). The NOE distance constraints were collected from the 100 ms mixing time ROESY spectrum recorded at 4°C in H_2O with a time domain data matrix of 512*2048 points corresponding to the t_{1max} and t_{2max} being 43 ms and 170 ms respectively. Zero-quantum coherences were suppressed as described in literature (Otting et al., J. Magn. Reson. 1990, 89, 423-430). Scalar spin-spin coupling constants $^3J_{H_\alpha H_\beta}$ and $^3J_{H_N H_\alpha}$ were measured by line-fitting from the 1D spectra. All spectra were baseline corrected by subtraction of suitable polynomials using the standard processing software provided by the spectrometer manufacturer.

The strategy followed for the structural determination of peptides was similar to that used for the structure determination of the PEC-60 (Liepinsh et al., J. Mol. Biol. 1994, 239, 137-153). The cross-peaks in the ROESY

spectra were assigned and integrated using the program EASY (Eccles et al., J. Biomol. NMR 1991, 1, 111-130). The ROESY cross-peak intensities were translated into upper bounds on the ^1H-^1H-distances with the program CALIBA (Güntert et al., J. Mol. Biol. 1991, 217, 517-530). The volumes of the cross-peaks between backbone protons including β-protons were converted into upper distance bounds, b, using a $1/b^6$ dependence, whereas $1/b^5$ dependency was used to obtain distance constraints from intra-residual NOEs with side-chain protons beyond the β-protons (Güntert et al., J. Mol. Biol. 1991, 217, 517-530; Güntert et al., J. Mol. Biol. 1991, 217, 531-540). The intra-residual and sequential NOE distance constraints together with the coupling constants $^3J_{H_\alpha H_\beta}$ and $^3J_{H_N H_\alpha}$ were used as input for the program HABAS (Güntert et al., J. Mol. Biol. 1991, 217, 517-530) to obtain stereo-specific assignments for β-methylene protons and constraints for the dihedral angles ϕ, ψ, χ_1.

The structure calculations were performed with the program DIANA (Güntert et al., J. Mol. Biol. 1991, 217, 517-530), using the REDAC strategy (Güntert and Wüthrich, J. Biomol. NMR, 1991, 1, 447-456) for improved convergence. The DIANA calculations were started using the angle constraints which were generated by HABAS from the combined data on coupling constants and NOEs. The initially calculated conformers were analysed using the program GLOMSA which compares the local geometry in the conformers with the NMR constraints to obtain further stereo-specific resonance assignments (Güntert et al., J. Mol. Biol. 1991, 217, 517-530; Güntert et al., J. Mol. Biol., 1991, 217, 531-540). We obtained stereo-specific assignments for Gly^2 α-protons and Cys^6, D-Nal^3, Arg^4 and Trp^5 β-protons, as well as Arg^4 γ-protons, in this way. The Compound Q1 structure was calculated using 147 constraints: 122 meaningful upper limit constraints, 22

angle constraints from HABAS and 3 constraints that fixed the SS bond. The final conformers were analysed using the program XAM (Xia: Software for determination and visual display of NMR structures of proteins: the distance geometry program DGPLAY and the computer graphics programs CONFOR and XAM. 1992, Ph. D. thesis No. 9831, ETH Zürich, Switzerland).

Results

Chemical shifts and measured coupling constants of Compound Q1 are presented in Tables 1 and 2, respectively. In Fig. 3 the protons are indexed, the indexed protons corresponding to the indices for protons given in Tables 1 and 2.

The 3D structure of Compound Q1 obtained from the NMR-studies is depicted in Fig. 4. Very interestingly, as is shown in the Figure, an unexpected hydrogen bonding between C=O of Nal^3 and -NH of Cys^6 was present in Compound Q1, indicating that Compound Q1 has a unique novel structure. Besides yielding a structure that might have unique pharmacological properties, the presence of intermolecular hydrogen bonding is expected to ease the passage through the blood-brain barrier due to increased hydrophobicity of the compound, as well as being taken up systemically after per oral administration.

Table 1. ^1H chemical shifts (± 0.01ppm) in Compound Q1

Cys[1]	H_N	H_α	$H_{\beta1}$	$H_{\beta2}$	H_γ	
	8.52	4.63	2.96	3.02	2.03	
Gly[2]	H_N	$H_{\alpha1}$	$H_{\alpha2}$			
	8.47	3.83	4.28			
Nal[3]	H_N	H_α	$H_{\beta1}$	$H_{\beta2}$	$H_{\delta1}$	$H_{\delta2}$
	8.45	4.54	3.01	3.30	7.37	7.62
	$H_{\varepsilon1}$	H_ζ	$H_{\eta1}$	$H_{\eta2}$	H_ι	
	7.86	7.80	7.84	7.48	7.46	
Arg[4]	H_N	H_α	$H_{\beta1}$	$H_{\beta2}$	$H_{\gamma1}$	$H_{\gamma2}$
	8.07	3.65	0.57	0.78	-0.09	0.12
	$H_{\delta1}$	$H_{\delta2}$	H_ε			
	2.05	2.12	6.43			
Trp[5]	H_N	H_α	$H_{\beta1}$	$H_{\beta2}$	$H_{\delta1}$	$H_{\varepsilon2}$
	8.05	4.68	3.29	3.38	7.20	10.15
	$H_{\varepsilon3}$	$H_{\zeta2}$	$H_{\zeta3}$	$H_{\eta2}$		
	7.62	7.40	7.09	7.16		
Cys[6]	H_N	H_α	$H_{\beta1}$	$H_{\beta2}$	$H_{\gamma1}$	$H_{\gamma2}$
	7.95	4.65	2.98	3.09	7.27	7.37

Table 2. Coupling constants $^3J(^1H-^1H)$ (± 1.0Hz) in Compound Q1

Cys^1	H_N	H_α	7.0
Cys^1	H_α	$H_{\beta1}$	7.5
Cys^1	H_α	$H_{\beta2}$	7.2
Gly^2	H_N	$H_{\alpha1}$	3.4
Gly^2	H_N	$H_{\alpha2}$	6.9
Nal^3	H_N	H_α	6.0
Nal^3	H_α	$H_{\beta1}$	12.0
Nal^3	H_α	$H_{\beta2}$	3.5
Arg^4	H_N	H_α	5.9
Arg^4	H_α	$H_{\beta1}$	12.0
Arg^4	H_α	$H_{\beta2}$	4.0
Trp^5	H_N	H_α	7.4
Trp^5	H_α	$H_{\beta1}$	12.0
Trp^5	H_α	$H_{\beta2}$	5.0
Cys^6	H_N	H_α	8.4
Cys^6	H_α	$H_{\beta1}$	12.0
Cys^6	H_α	$H_{\beta2}$	4.0

Example 5

Effect of Compound Q1 on feeding behaviour in rats after intraperitoneal administration

Animals

Male Wistar rats (National Laboratory Animal Center, Kuopio, Finland) weighing 290-320 g at the time of surgery, were housed individually in hanging wire mesh cages (45x37x19 cm) with free access to food and water in a temperature controlled room at 20±1°C with a 12:12 h light:dark cycle (lights on at 08.00 h). The rats had free access to food pellets and tap water.

Experimental protocol and injection of Compound Q1

On the day of the experiment, the food was removed from wire baskets and the rats were injected intra-peritoneally (i.p.) with vehicle (saline) or Compound Q1. Rats were returned to their home cage and 7 pre-weighed pellets (20-25g) were presented on clean plastic Petri dishes. All injections were carried out between 12.00-13.00 every third day and were given in randomised order in such a way that none of the rats received the same dose of Compound Q1 twice. Food intake was measured after 1, 2, and 4 h following the i.p. injection by weighting remaining pellets and spillage using Mettler balance to the nearest 0.1g.

Statistical evaluation

All results are expressed as mean±SEM. The cumulative food intake data and the amount of food consumed during specific time periods were analysed by one way analysis of variance (ANOVA) for repeated measures, followed by multiple comparisons using LSD test where it was appropriate.

Results

The results are summarized in Fig. 5 and show that 0.1 mg/kg and 0.5 mg/kg doses of Compound Q1, injected intraperitoneally, increased food intake after two hours from the injection. This increase was significant for both of the doses ($p<0.05$) after 4 hours compared to the saline control. The increase in food intake was approximately 48% (0.5 mg/kg) after 4 hours compared with the basal food intake.

These results show that Compound Q1 is capable of penetrating the blood-brain barrier and exerting an antagonistic action in the central nervous system.

A number of novel compounds were synthesised in an analogous way to the synthesis of Compound Q1, described in Example 1. If another synthetic route was used, the description is given in the Example.

Example 6. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, D-Phe^7, L-Cys-NH$_2$^{10}) α -MSH$_{5-10}$ trifluoroacetate (Compound Q2, SEQ ID NO:6)* was made essentially as described in Example 1. Yield 18%. Rf 0.65. k' 2.0(13% MeCN in 0.1% TFA). m/e 811.0.

Example 7. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, D-diClPhe^7, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate (Compound Q3, SEQ ID NO:7)* was made essentially as described in Example 1. Yield 30%. Rf 0.69. k' 4.6(23% MeCN in 0.1% TFA). m/e 879.6.

Example 8. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, L-Nal^7, L-Cys-NH$_2$^{10}) α -MSH$_{5-10}$ trifluoroacetate (Compound Q4, SEQ ID NO:8)* was made essentially as described in Example 1. Yield 35%. Rf 0.53. k' 3.2(24% MeCN in 0.1% TFA). m/e 861.

Example 9. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, L-Leu^7, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate (Compound Q5, SEQ ID NO:9)* was made essentially as described in Example 1. Yield 29%. Rf 0.55 . k' 2.6(20% MeCN in 0.1% TFA). m/e 776.9.

Example 10. *Synthesis of cyclo(S-S)-(Ac-L-Pen^5, Gly^6, D-Nal^7, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate (Compound Q6, SEQ ID NO:10)* was made essentially as described in Example 1. Yield 29%. Rf 0.68. k' 2.0(24% MeCN in 0.1% TFA). m/e 888.5.

Example 11. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, D-Nal^7, L-Pen-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate (Compound Q7, SEQ ID NO:11)* was made essentially as described in Example 1. Yield 24%. Rf 0.73. k' 4.6(24% MeCN in 0.1% TFA). m/e 888.6.

Example 12. *Synthesis of cyclo(S-S)-(Ac-L-Pen^5, Gly^6, D-Nal^7, L-Pen-NH$_2$^{10}) α -MSH$_{5-10}$ trifluoroacetate (Compound Q8, SEQ ID NO:12)* was made essentially as described in Example 1. Yield 14%. Rf 0.77. k' 4.6(27% MeCN in 0.1% TFA). m/e 917.1.

Example 13. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, L-Ala^6, D-Nal^7, L-Cys-NH$_2$^{10}) α -MSH$_{5-10}$ trifluoroacetate (Compound Q9, SEQ ID NO:13)* was made essentially as described in Example 1. Yield 12%. Rf 0.76. k' 3.8(29% MeCN in 0.1% TFA). m/e 874.6.

Example 14. *Synthesis of cyclo(S-S)-(L-Cys^5, Gly^6, D-Nal^7, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate (Compound Q10, SEQ ID NO:14)* was made essentially as described in Example 1. Yield 20%. Rf 0.55. k' 3.5(17% MeCN in 0.1% TFA). m/e 818.6.

Example 15. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, D-Nal(1)^7, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate (Compound Q11, SEQ ID NO:15)* was made essentially as described in Example 1. Yield 29%. R_f 0.64. k'5.8(23% MeCN in 0.1% TFA). m/e 860.6.

Example 16. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, L-Nal(1)^7, L-Cys-NH$_2$^{10}) α -MSH$_{5-10}$ trifluoroacetate (Compound Q12, SEQ ID NO:16)* was made essentially as described in Example 1 Yield 40 %. R_f 0.56. k'6.7(24% MeCN in 0.1% TFA). m/e 861.3.

Example 17. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, D-Nal^7, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ ditrifluoroacetate (Compound Q13, SEQ ID NO:17)* was made essentially as described in Example 1. Yield 7.2 mg(30 %). R_f 0.51(1-butanol - AcOH - water, 4:1:1).k' 2.1(11% MeCN in 0.1% TFA). m/e 940.8.

Example 18.

Synthesis of cyclo-(Aoa^6, D-Nal^7) α-MSH$_{6-9}$ trifluoracetate (Compound Q14, SEQ ID NO:18).

330 mg (0.073 mmole) of TentaGel S Trt-Trp(Boc)Fmoc (*Rapp polymere*) was placed into the peptide synthesis column. Then the Fmoc group was removed by 20% piperidine in DMF (5 min), support washed with DMF. Fmoc-Arg(Pbf)-OH (141 mg, 0.22 mmole), TBTU (70 mg, 0.22 mmole) and DIEA(0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-D-Nal-OH (96 mg, 0.22 mmole), TBTU (70 mg, 0.22 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through

the column for 60 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-Aoa-OH (84 mg, 0.22 mmole), TBTU (70 mg, 0.22 mmole) and DIEA(0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 0.3 M Ac_2O in DMF for 5 min, washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again with DMF, then methanol, then dichloromethane. Then the peptidylpolymer obtained was placed into a glass column (20 x 110 mm) with a sintered glass layer and stopcock at the bottom. A 5 ml portion of a mixture TFA - TIS - CH_2Cl_2 (2:2:96) was added, allowed to stand for 5 min at room temperature, then under vacuum filtered into a flask containing a stirred solution of Na acetate trihydrate (1.2 g) in water. Then the next portion of TFA solution was added to the polymer, the above treatment repeated and it was filtered into the same flask. So it was repeated 5 times. Then the lower layer from the filtering flask was separated, placed at -20°C for 10 hours, ice separated removed by filtration, filtrate dried over $MgSO_4$. It was filtered again, filtrate evaporated.

The residue was dissolved in 20 ml MeOH. This solution was twice slowly passed through a column (5.5 x 50 mm) with Dowex 1(*Sigma*) in Cl^- form. Then it was evaporated, and the residue treated with dry ether. A crystalline precipitate formed was filtered off. Yield of the linear precursor NH_2-$(CH_2)_7$CO-D-Nal-Arg(Pbf)-Trp(Boc)-OH.HCl was 52 mg (66%). Linear precursor (52 mg, 0.048 mmol) was dissolved in 25 ml DMF, cooled to 0°C, N-methylmorpholine (10.8 ml, 0.096 mmol) and DPPA (23.5 ml, 0.096 mmol) added. It was allowed to stand at 0°C for 2 days, additionally 10.8 ml NMM added, then it was allowed to

stand at 0°C for 2 days again. Then the mixture was evaporated, the residue triturated with ether. The crystalline precipitate formed was filtered off, washed on the filter with dry ether, then washed with 5% aqueous $NaHSO_4$, water, 5% aqueous $NaHCO_3$, water again, then dried in vacuum in the presence of P_2O_5. The obtained protected cyclopeptide cyclo-/NH-$(CH_2)_7$CO-D-Nal-Arg(Pbf)-Trp(Boc)/ was dissolved in 2 ml of deprotection mixture (TFA - water - 1,2- ethanedithiol - TIS, 92.5:2.5:2.5:2.5) and allowed to stand at room temperature for 3 hours. Then it was evaporated at 0°C, dry ether was added, the precipitate formed was filtered off and washed on the filter with ether, then dried *in vacuo* over KOH. The raw product obtained was dissolved in 0.5 ml of 60 % MeCN in water, solution divided into 3 portions and placed into centrifuge tubes, each of them was diluted with 0.1 % aqueous TFA to 1.5 ml volume. It was centrifuged and the clear solutions applied onto an HPLC semi-preparative column (10 x 250 mm, Vydac RP C_{18} , 90 A, 201HS1010), eluate - 23% MeCN in water + 0.1% TFA, detection at 220 nm. Eluate fractions containing pure putative Compound Q14 were pooled and lyophilized. A white powder formed. Yield 12.4 mg(21 %). R_f 0.82. k' 3.3(23% MeCN in 0.1% TFA). m/e 682.

Example 19. *Synthesis of cyclo-(Aua^6, D-Nal^7) α-MSH$_{6-9}$ trifluoroacetate (Compound Q15, SEQ ID NO:19)* was made essentially as described in Example 18. Yield 19%. R_f 0.81. k' 4.9(28% MeCN in 0.1% TFA). m/e 723.9.

Example 20. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, D-NMePhe^7, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate (Compound Q16, SEQ ID NO:20)* was made essentially as described in Example 1. Yield 42%. R_f 0.62. k' 1.8(12% MeCN in 0.1% TFA). m/e 825.0.

Example 21.

Synthesis of cyclo(Asp βCO---->Lys εNH)-(Ac-L-Lys^6, D-Nal^7, L-Asp-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoracetate (Compound Q17, SEQ ID NO:21).

The peptide sequence was assembled on a solid support using "Pioneer" peptide synthesis system. Fmoc-PAL-PEG-PS (333 mg, 0.05 mmole) was placed into the peptide synthesis column. Then the Fmoc group was removed by 20% piperidine in DMF (5 min), support washed with DMF. Fmoc-Asp(OAl)-OH (59 mg, 0.15 mmole), HATU (53 mg, 0.15 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 30 min. Then the support was washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-Trp(Boc)-OH (79 mg, 0.15 mmole), HATU (53 mg, 0.15 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 30 min. Then the support was washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-Arg(Pbf)-OH (97 mg, 0.15 mmole), HATU (53 mg, 0.15 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-D-Nal-OH (66 mg, 0.15 mmole), HATU (53 mg, 0.15 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again. Fmoc-Lys(Aloc)-OH (59 mg, 0.15 mmole), HATU (53 mg, 0.15 mmole) and DIEA (0.17 ml, 1.0 mmole) were dissolved in 4 ml DMF and circulated through the column for 60 min. Then the support was washed with DMF, treated with 20% piperidine in DMF (5 min), and washed again; then treated with 0.3 M Ac_2O in DMF for 5 min and washed with DMF.

Then the support was washed with 5% AcOH + 2.5% NMM in chloroform. Tetrakis(triphenylphosphine)-palladium(0) (173 mg, 0.15 mmol) was dissolved in 4 ml of the abovementioned mixture and circulated through the column for 2 hours. The support was washed with 0.5% DIEA + 0.5% Na diethyldithiocarbamate in DMF, then it was washed with pure DMF. PyAOP (78 mg, 0.15 mmol) was dissolved in 4 ml DMF and circulated through the column for 8 hours. Then it was washed with DMF, then methanol, then dichloromethane and dried *in vacuo*. The resin was treated with 5 ml of deprotection mixture (TFA - water - 1,2-ethanedithiol - TIS, 92.5:2.5:2.5:2.5) and allowed to stand at room temperature for 3 hours. Then it was evaporated at 0°C, dry ether was added, the precipitate formed was filtered off and washed on the filter with ether, then dried in vacuo over KOH. The raw product obtained was dissolved in 0.5 ml of 60 % MeCN in water, solution divided into 3 portions and placed into centrifuge tubes, each of them was diluted with 0.1 % aqueous TFA to 1.5 ml volume. It was centrifuged and the clear solutions applied onto an HPLC semipreparative column (10 x 250 mm, Vydac RP C_{18} , 90 A, 201HS1010), eluate - 22% MeCN in water + 0.1% TFA, detection at 220 nm. Eluate fractions, containing pure putative Compound Q17 were pooled and lyophilized. A white powder formed. Yield 30.8 mg(17 %). R_f 0.54. k' 2.5(22% MeCN in 0.1% TFA). m/e 824.8.

Example 22. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, D-Nal^7, L-Orn^8, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate (Compound Q18, SEQ ID NO:22)* was made essentially as described in Example 6. Yield 23%. R_f 0.63. k' 7.7(20% MeCN in 0.1% TFA). m/e 832.9.

Example 23. *Synthesis of cyclo(S-S)-(Ac-L-Cys^5, Gly^6, D-Nal^7, L-Lys^8, L-Cys-NH$_2$^{10}) α-MSH$_{5-10}$ trifluoroacetate*

(Compound Q19, SEQ ID NO:23) was made essentially as described in Example 1. Yield 10%. R_f 0.63. k' 6.3 (24% MeCN in 0.1% TFA). m/e 818.7.

Example 24. *Synthesis of cyclo(S-S)-(Ac-D-Cys-D-Trp-D-Arg-Nal-Gly-D-Cys-NH_2) trifluoroacetate (Compound Q20, SEQ ID NO:24)* was made essentially as described in Example 1. Yield 13 %. R_f 0.90. k' 4.1 (17% MeCN in 0.1% TFA). m/e 861.

Example 25.

Assay of binding affinities of compounds of the invention for human MC-receptors

Expression of receptor clones. Human MC1- and MC5-receptor DNAs (Chhajlani and Wikberg, FEBS Lett. 1992, 309, 417-420; Chhajlani et al., Biochem. Biophys. Res. Commun. 1993, 195, 866-873), cloned into the expression vector pRc/CMV (InVitrogen Corp., USA), and human MC3 and human MC4-receptor DNAs (Gantz et al., J. Biol. Chem. 1993, 268, 8246-8250; Gantz et al., J. Biol. Chem. 1993, 268, 15174-15179), cloned into the expression vector pCMV/neo, were used. COS cells were grown and transfected with receptor clones as described (Schiöth et al., Eur. J. Pharmacol., Mol. Pharm. Sect. 1995, 288, 311-317; Schiöth et al., Pharmacol. Toxicol. 1996, 79, 161-165). After transfection cells were cultivated for 48 h, detached from the petri dishes, and used for radio-ligand binding as described (Schiöth et al., Eur. J. Pharmacol., Mol. Pharm. Sect. 1995, 288, 311-317; Schiöth et al., Pharmacol. Toxicol. 1996, 79, 161-165).

Binding studies. The transfected cells were washed with binding buffer (Minimum Essential Medium with Earle's salts, 25 mM HEPES, pH 7.0, 0.2 % bovine serum albumin

and distributed into 96 well plates. The cells were then incubated for 2 h at 37°C, with 0.1 ml binding buffer in each well containing [^{125}I][Nle^4, D-Phe^7]α-MSH and appropriate concentrations of the peptide to be tested. After incubation the plates were put on ice and the cells were washed with 0.1 ml of ice-cold binding buffer. The cells were then detached from the plates with 0.2 ml of 0.1 N NaOH. Radioactivity was counted by using a Wallac, Wizard automatic gamma counter. The competition data were analysed by fitting it to the logistic function using non-linear regression analysis. The K_i-values were then calculated from the thus obtained IC50-values by using the Cheng and Prusoff equation, essentially as described (Schiöth et al., Eur. J. Pharmacol., Mol. Pharm. Sect. 1995, 288, 311-317; Schiöth et al., Pharmacol. Toxicol. 1996, 79, 161-165).

Results. The K_i-values of the compounds of the invention for the human MC1, MC3, MC4 and MC5 receptors were as follows:

	MC1	MC3	MC4	MC5
Compound	K_i (nM)	K_i (nM)	K_i (nM)	K_i (nM)
Q1	3,543	228	12.4	5,118
Q2	14,568	23,717	1,374	41,059
Q3	2,936	642	106	17,630
Q4	>1,000,000	17,926	34,925	16,315
Q5	>1,000,000	>1,000,000	>1,000,000	>1,000,000
Q6	4,062	831	57	15,000
Q7	7,604	767	326	10,884
Q8	>1,000,000	1,158	175	5,701
Q9	7,840	637	22	4,881
Q10	6,304	1,103	74	2,380
Q11	>1,000,000	17,338	6,068	25,442
Q12	>1,000,000	16,447	32,482	13,216
Q13	1,398	430	291	1,603
Q14	7,354	31,454	19,704	17,962
Q15	112,549	13,624	7,330	11,693
Q16	>1,000,000	>1,000,000	53,430	>1,000,000
Q17	5,490	10,812	1,020	45,593
Q18	>1,000,000	5,016	2,143	95,062
Q19	>1,000,000	20,333	3,951	67,401
Q20	3,448,276	1,008,065	980,392	990,099

Example 26

Demonstration of the MC-receptor blocking capacity of Compound Q1

Expression of receptor clones.

Human MC1- and MC5-receptor DNAs (Chhajlani and Wikberg, FEBS Lett. 1992, 309, 417-420; Chhajlani et al., Biochem. Biophys. Res. Commun. 1993, 195, 866-873), cloned into the expression vector pRc/CMV (InVitrogen Corp., USA), and human MC3 and human MC4-receptor DNAs

(Gantz et al., J. Biol. Chem. 1993, 268, 8246-8250; Gantz et al., J. Biol. Chem. 1993, 268, 15174-15179), cloned into the expression vector pCMV/neo, were used. COS cells were grown and transfected with receptor clones as described (Schiöth et al., Eur. J. Pharmacol., Mol. Pharm. Sect. 1995, 288, 311-317; Schiöth et al., Pharmacol. Toxicol. 1996, 79, 161-165). After transfection cells were cultivated for 48 h, detached from the petri dishes, and used for cAMP measurements as described in the next paragraph.

Incubation of cells

The cells were detached from 60-80% confluent adherent cultures using Hank's balanced salts containing 0.5 mM EDTA and incubated for 30-60 min at 37°C in ordinary growth medium containing 0.5 mM of the phosphodiesterase inhibitor 3-*iso*-butyl-1-methyl-xanthine (IBMX). 20 µl aliquots of appropriate dilutions of Compound Q1 and α-MSH in growth medium were prepared in 96 well microtitre plates and placed in a water bath at 37°C. About $1.5x10^5$ cells in 180 µl were thereafter quickly added to each well to obtain immediate mixing. After 20 min 20 µl of 4.4 M perchloric acid were added, mixed, neutralized after a few minutes by addition of 20 µl base (5 M KOH, 1 M Tris) and centrifuged.

Determination of cAMP

20 µl of acid treated supernatant obtained above were mixed with 50 µl buffer (100 mM Tris-Cl, 250 mM NaCl, 10 mM EDTA, 0.1% mercaptoethanol, 0.5 mM IBMX, pH=7.4) containing 0.01 µCi [^{3}H]cAMP (Amersham, 1.04 TBq/mmol, 1 µCi/µl, product no.: TRK304). 200 µl of the same buffer containing a 1:16 diluted porcine adrenal gland bark extract (prepared as described by Nordstedt and Fredholm,

Anal. Biochem., 1990, 189, 8258-8262) were added and the microtitre plates were incubated for at least 2 hours at 4°C. A standard curve was prepared in the same manner with dilutions of cAMP covering the range 0.5 nM - 2 μM.

After completion of incubation the solutions were filtered over GF-B glassfibre filters (Whatman) and washed briefly with ca. 2 ml ice-cold washing buffer (50 mM Tris-Cl, pH= 7.4). Radioactivity on the filters was measured after addition of scintillation liquid. Stimulation experiments were determined in quadruplicates and standard curves in duplicates.

Results

The results are shown in Figs. 6 and 7. As can be seen from the Figures α-MSH 10^{-12} - 10^{-5} M caused a dose dependent increase of cAMP in all of the human MC1, MC3, MC4 and MC5 receptor expressing cells (hMC1, hMC3, hMC4 and hMC5, respectively). However, in the presence of 100 nM of Compound Q1 the α-MSH response on cAMP was essentially completely blocked in the hMC1, hMC3 and hMC4 receptor expressing cells. For the hMC5 receptor expressing cells the α-MSH response on cAMP was also powerfully inhibited, although at concentrations above 10^{-8} M of α-MSH residual stimulation of cAMP was still seen. These results thus indicate the MSH-receptor blocking capacity of Compound Q1, as well as the agouti mimetic capacity of Compound Q1 on MC1 and MC4 receptors.

Example 27

Effects of Compound Q1 on withdrawal intensity in opioid dependent rats.

Adult male Sprague-Dawley rats were used throughout the

experiment.

One group of rats were treated with saline, whereas the remaining 5 groups (groups 2-6) of rats received morphine at a dose of 10 mg/kg sc, for a week.

On the 8th day, rats in the control group received saline 1 hour before naloxone was administered (2 mg/kg) and behaviour was studied. Group 2 received saline prior to naloxone; groups 3 and 4 received Compound Q1 (0.1 and 0.5 mg/kg sc, respectively); and groups 5 and 6 received GR82334 (an NKI receptor antagonist, 0.1 and 0.5 mg/kg sc, respectively) 1 hour before naloxone.

A number of behavioural effects were studied and the table below summarizes the results (mean ± SEM):

withdrawal sign	MSN	Q1	Q1	GR82334	GR82334
		0.5 mg/kg	0.1 mg/kg	0.5 mg/kg	0.1 mg/kg
rearing	22.4±4.7	26.5±3.2	28.4±2.7	21.1±2.3	27.8±2.8
wet dog shake	3.0±0.7	0.3±0.2*	2.4±1.0	1.9±0.4	1.3±1.0
escape jumping	1.1±0.8	1.7±0.6	2.4±1.0	2.4±1.0	0.7±0.6
face washing	2.6±0.4	3.2±0.6	2.8±0.8	3.9±0.8	2.8±0.9
grooming	1.6±0.8	1.2±0.5	1.2±1.0	1.7±0.6	1.8±0.9
teeth chattering	12.3±3.2	4.3±1.0*	7.0±2.8	7.3±1.3	10.3±2.3
paw tremble	0.6±0.3	0±0	0±0	0.7±0.5	0±0
digging	34.1±7.6	27.7±7.1	15±5.3	16.7±2.8	25.8±8.2
diarrhoea	0.6±0.2	0.2±0.2	0.6±0.2	0.4±0.2	0.5±0.2
chewing	4±0.8	2.7±1.1	1.4±1.0	3.1±0.8	2.3±1.1
scratching	0.7±0.4	0.2±0.2	0.6±0.2	0.1±0.1	0.8±0.5
ptosis	0.9±0.1	0.3±0.2*	0.4±0.2	0.7±0.2	1.0±0
stretch	3.0±3.0	0±0	1.0±0.4	0.4±0.2	0±0

* significant difference vs MSN, $p<0.05$, ANOVA

MSN = group 2 (Morphine for 7 days, Saline and Naloxone on day 8)

Compound Q1 = groups 3 and 4 (morphine for 7 days, Compound Q1 at different doses followed by naloxone on day 8)

GR82334 = groups 5 and 6 (morphine for 7 days, GR at different doses followed by naloxone on day 8)

(GR82334 is also known as physalemin, 9-deglycine-10-[(5S)-6-oxo-L-alpha-(2-methylpropyl)-1,7-diazaspiro[4,4]nonane-7-acetic acid]-11-L-tryptophanamide; CAS No. 129623-01-4)

5

The results clearly demonstrated significant improvement on the withdrawal behaviour with Compound Q1 in a dose dependent manner.

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receptors

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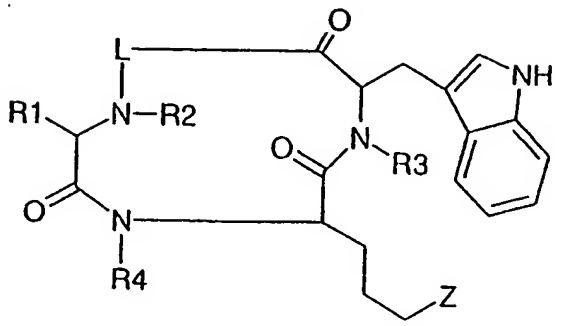
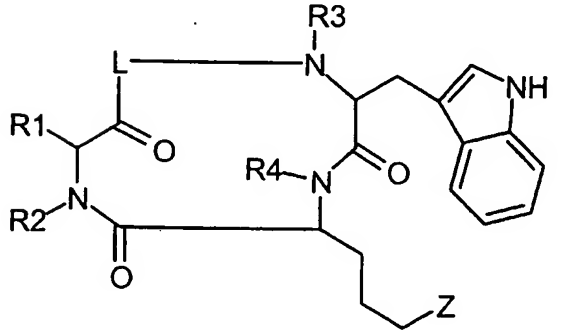
Claims

1. A compound of formula (1) or formula (2)



(1)



(2)

wherein L is a linking group so as to create a cycle which contains from 18 to 21 ring-atoms;

Z is selected from $-NH_2$ and guanidino;

R1 is $-CH_2X$ where X is selected from phenyl substituted with halogen, methyl, phenyl, methoxy, nitro, preferably in the 3 and/or 4 position, or 2-naphthyl or an aromatic system consisting of 3 fused benzene rings;

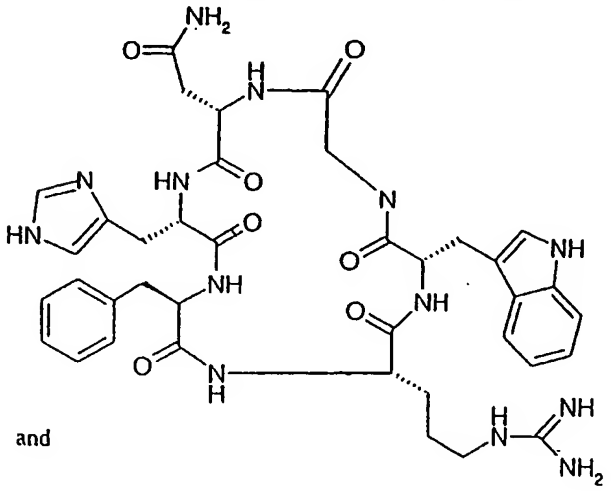
and

R2, R3 and R4 are selected from hydrogen and methyl, with hydrogen being preferred;

or a pharmaceutically-acceptable salt thereof;

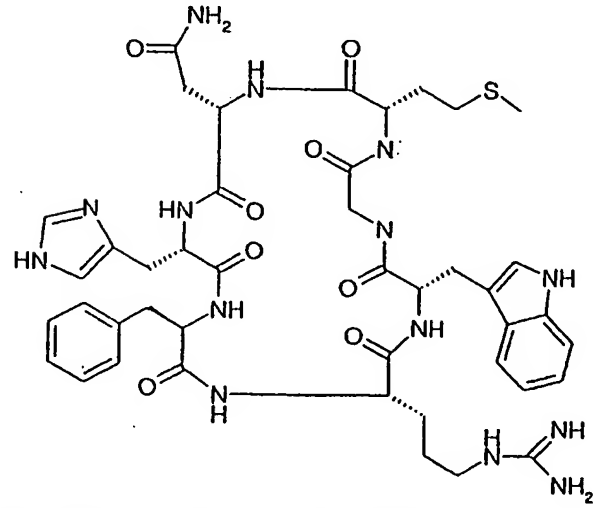
wherein

the compounds cNHdFRWG (SEQ ID NO:2) and cMNHdFRWG (SEQ ID NO:3), having structural formulae as follows



cNHdFRWG

and



cMNHdFRWG

are specifically excluded.

2. A compound as claimed in any one of the preceding claims wherein Z is guanidino.

3. A compound as claimed in claim 1 wherein R1 is $-CH_2X$ where X is 2-naphthyl.

4. A compound as claimed in any one of the preceding claims wherein the number of atoms in X exceeds 11.

5. A compound as claimed in any one of the preceding claims wherein the number of carbon atoms in X exceeds 6.

6. A compound as claimed in any one of the preceding claims wherein the number of heavy atoms in X exceeds 5.

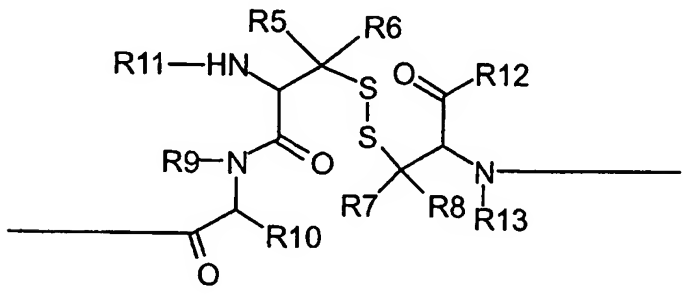
7. A compound as claimed in any one of the preceding claims wherein the mass of X exceeds 77.3 daltons.

8. A compound as claimed in any one of the preceding claims wherein L contains 20 ring-atoms.

9. A compound as claimed in any one of the preceding claims wherein L contains a disulphide bridge, the 2 connected sulphur atoms in this bridge being part of the ring.

10. A compound as claimed in any one of claims 1 to 7 wherein the linking group, L, is selected from

I



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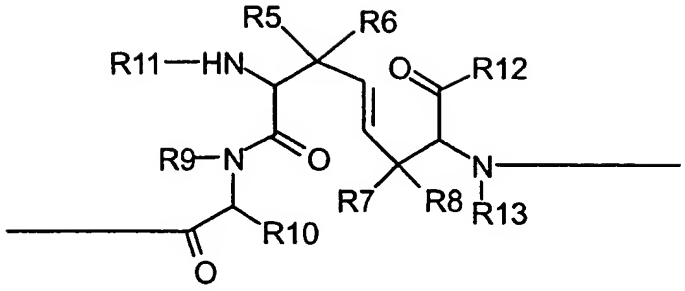

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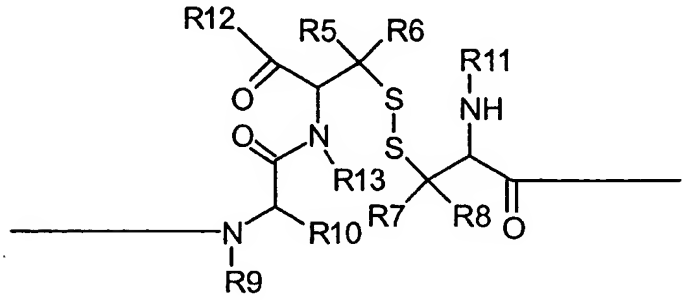

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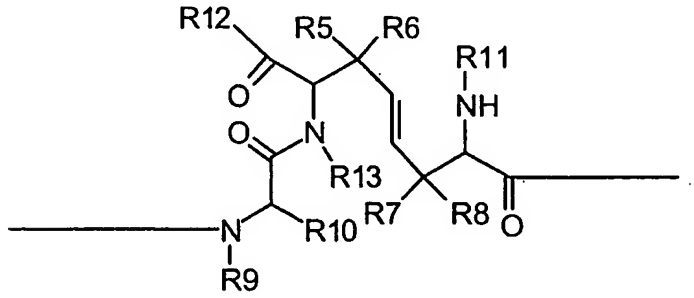


wherein R5, R6, R7, R8, R9 and R13 are selected from hydrogen and methyl;

R10 is selected from X, or $-CH_2X$ where X is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted

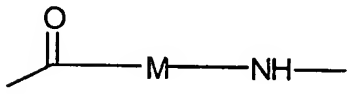
heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, or a functional group;

R11 is selected from H, acetyl, alkyl, amino-acid residue, amino-acid analogue residue, peptide residue and a functional group;

and

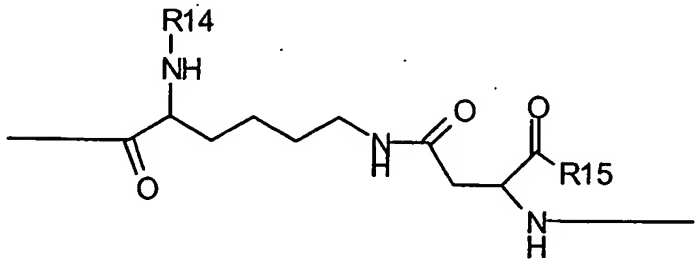
R12 is selected from hydrogen, $-NH_2$, hydroxy, methoxy, isopropoxy, alkyl, amino-acid residue, amino-acid analogue residue, peptide residue and a functional group.

V.



wherein M is a saturated or unsaturated linear hydrocarbon chain of 7 to 10 carbon atoms.

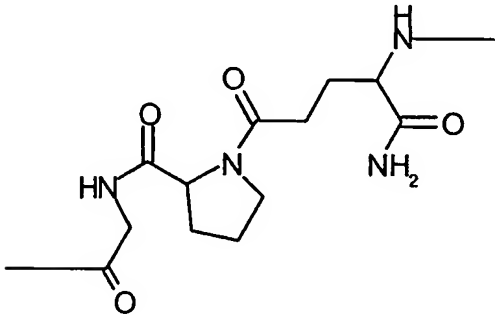
VI.



wherein R14 is selected from hydrogen, acyl, alkyl, amino-acid residue, amino-acid analogue residue, peptide residue and a functional group;

wherein R15 is selected from hydrogen, $-NH_2$, hydroxy, alkyl, methoxy, isopropoxy, amino-acid residue, amino-acid analogue residue, peptide residue and a functional group.

VII.



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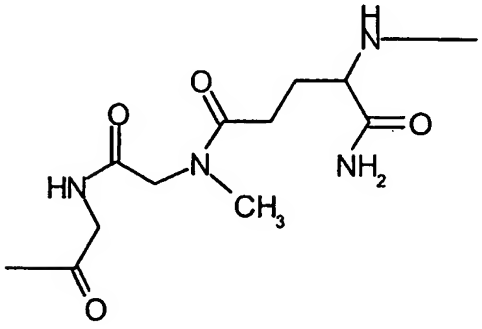


or

IX.

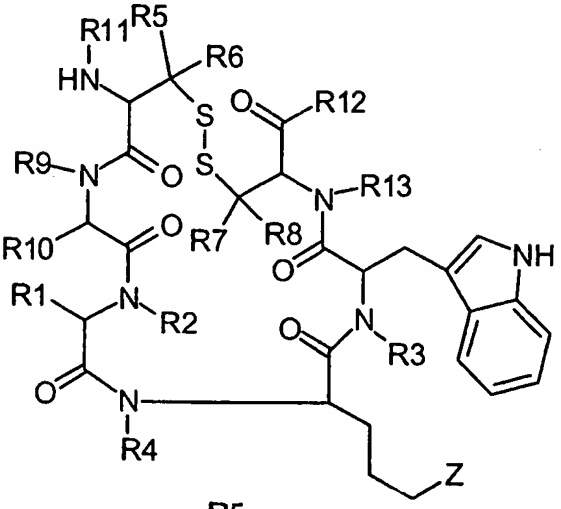
the linking group comprises

-Gly-Ala-Gly-

or

-Gly-Gly-Gly-Gly- (SEQ ID NO:4) or other peptide residues.

11. A compound as claimed in claim 10 of formula (3) or (4)



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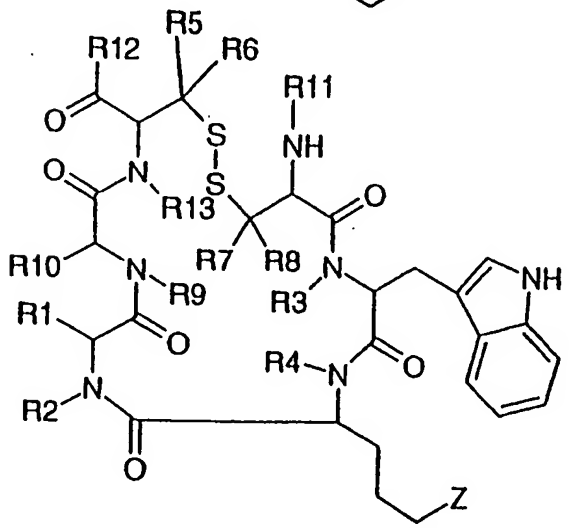


(4)

12. A compound as claimed in claim 10 or claim 11 wherein one or more of R2, R3, R4, R5, R6, R7, R8, R9 and R13 are hydrogen.

13. A compound as claimed in any one of claims 10 to 12 wherein R10 is H or methyl.

14. A compound as claimed in any one of claims 10 to 13 wherein R10 is selected so as to have less than 12 atoms.

15. A compound as claimed in any one of claims 10 to 14 wherein R10 is selected so as to have less than 5 carbon atoms.

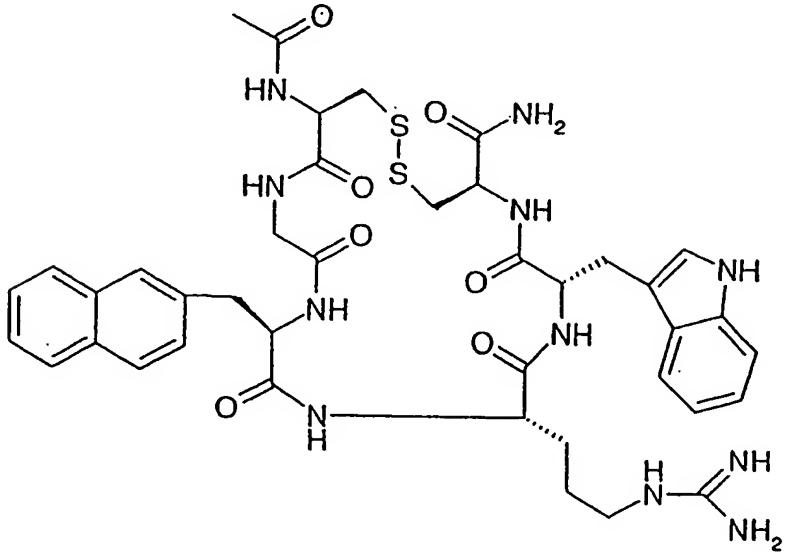
16. A compound as claimed in any one of claims 10 to 15 wherein R10 is selected so as to have less than 5 heavy atoms.

17. A compound as claimed in any one of claims 10 to 16 wherein R10 is selected so as to have a mass of less than 82 daltons.

18. A compound as claimed in any one of claims 10 to 17 wherein R11 is hydrogen or acetyl.

19. A compound as claimed in any one of claims 10 to 18 wherein R12 is $-NH_2$ or hydroxy.

20. A compound of formula Q1 (SEQ ID NO: 5)



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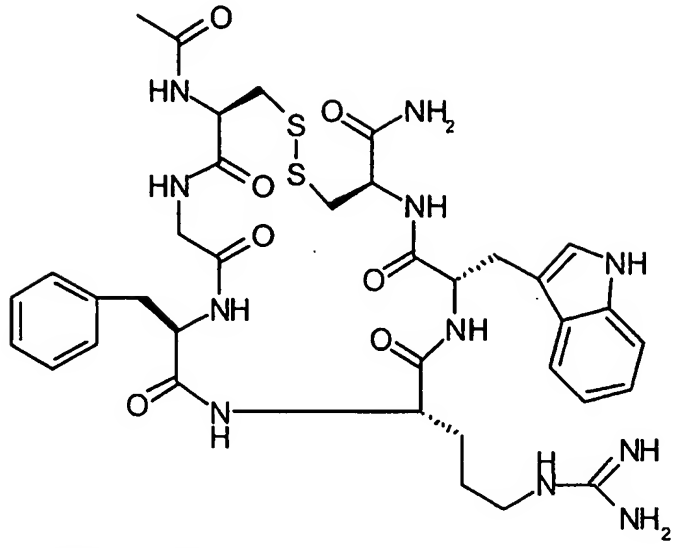

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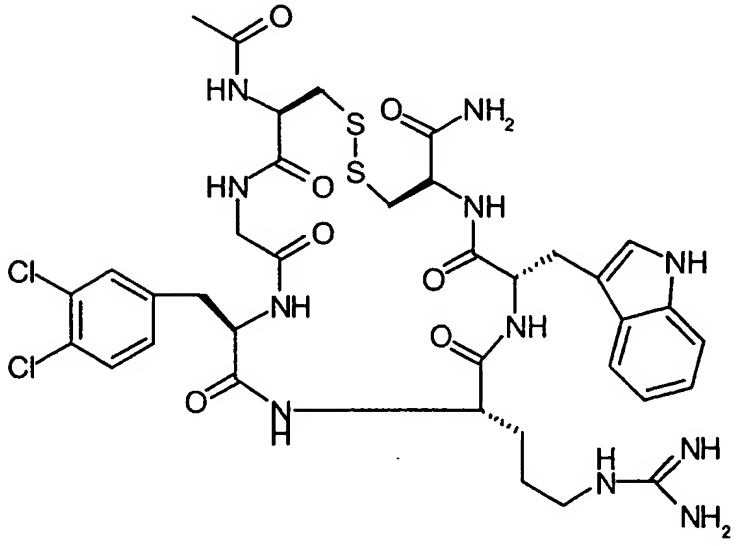

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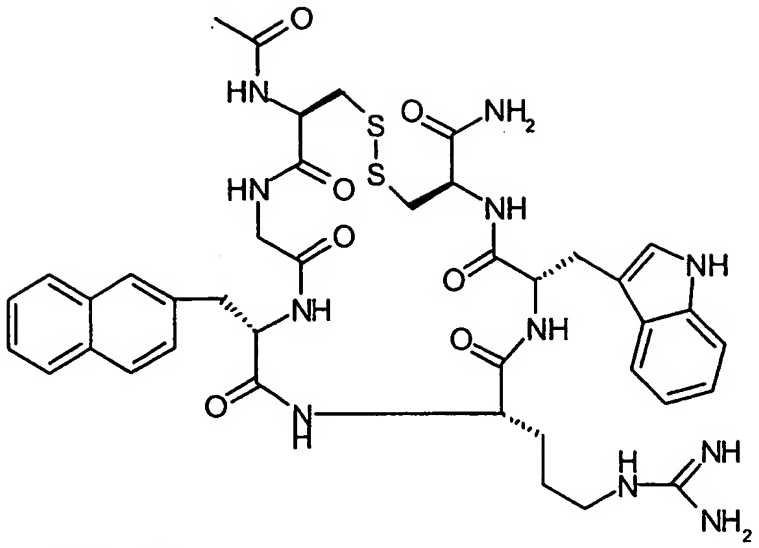

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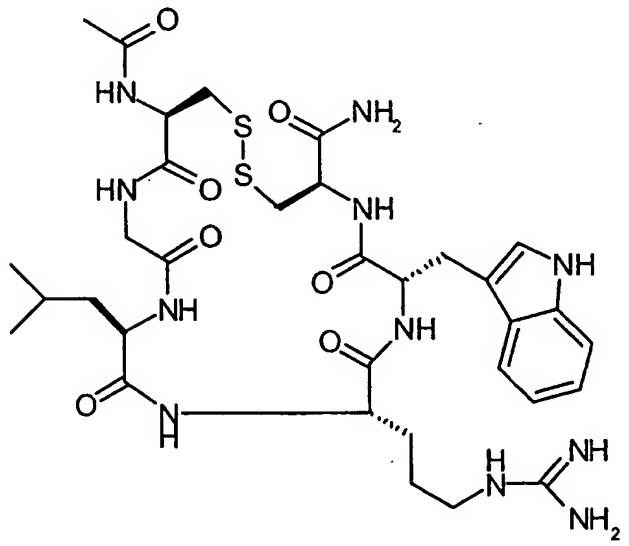

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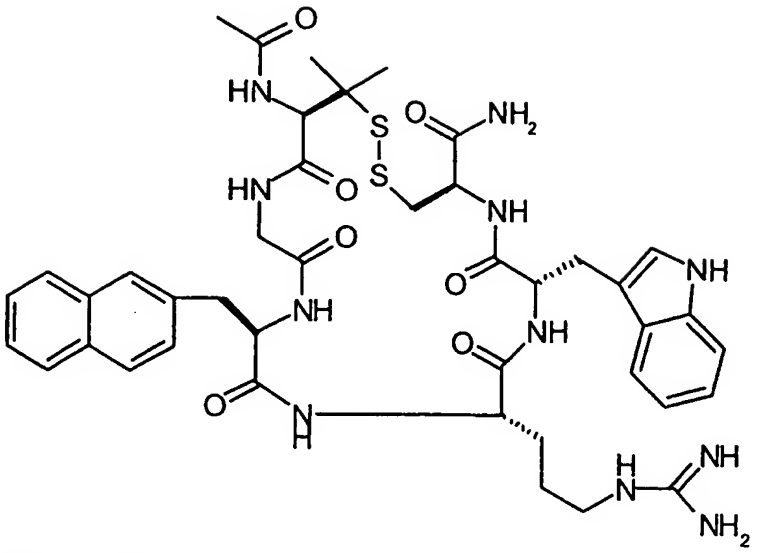

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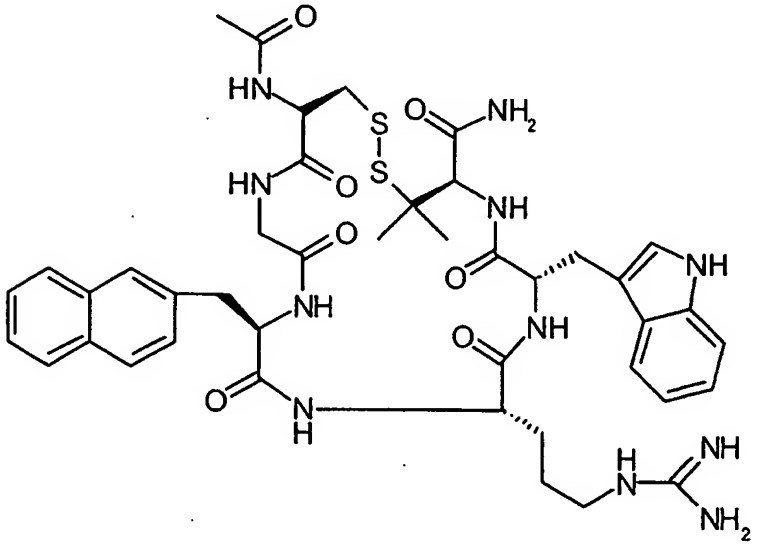

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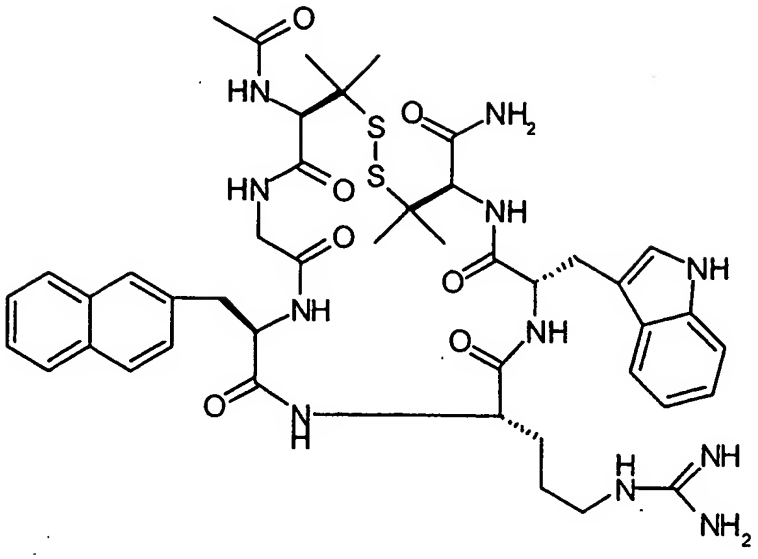

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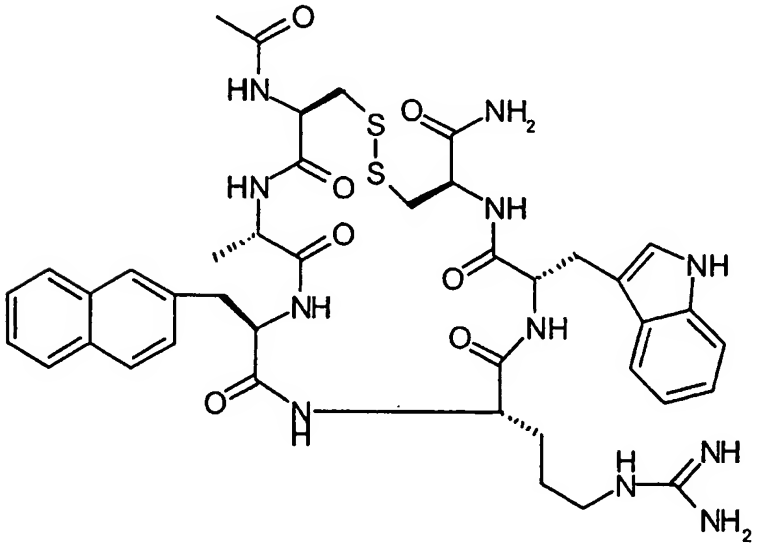

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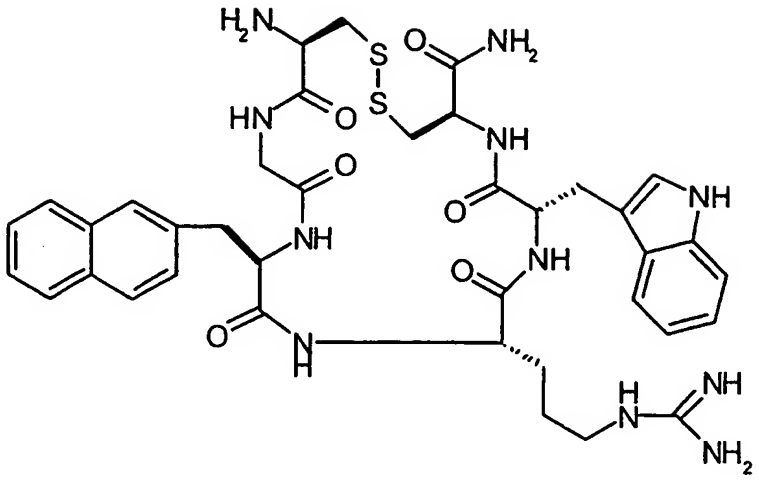

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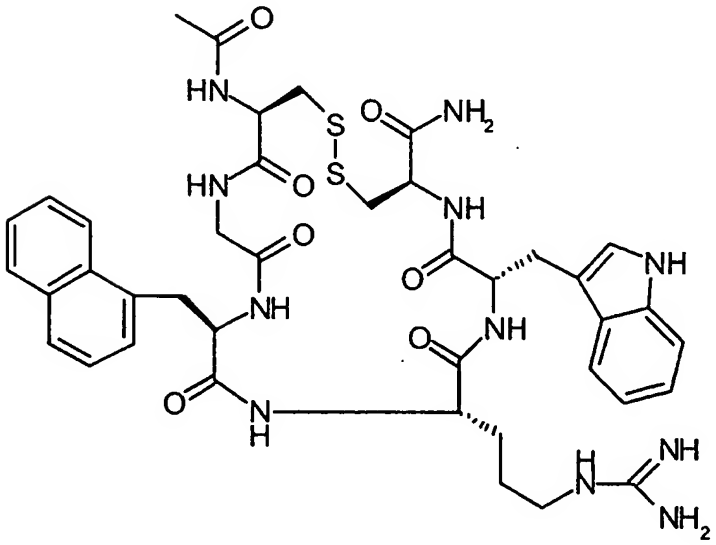

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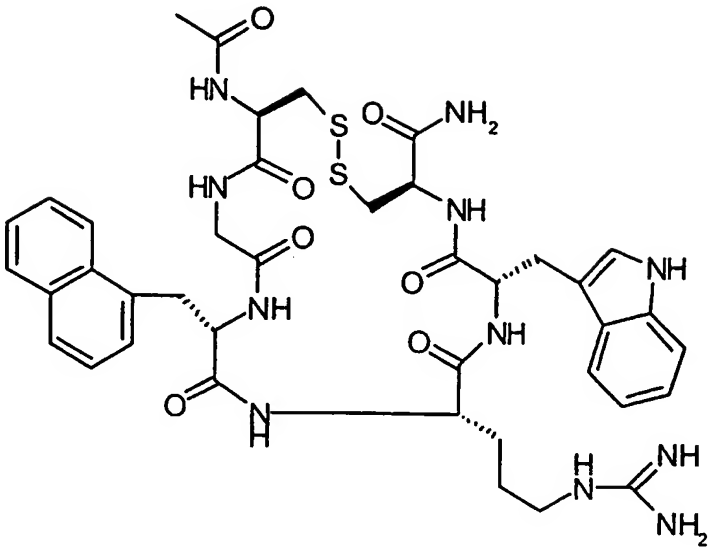

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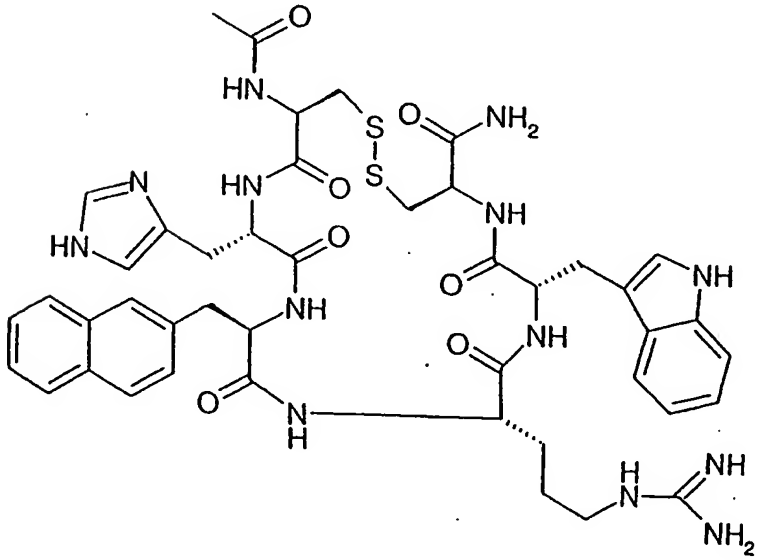

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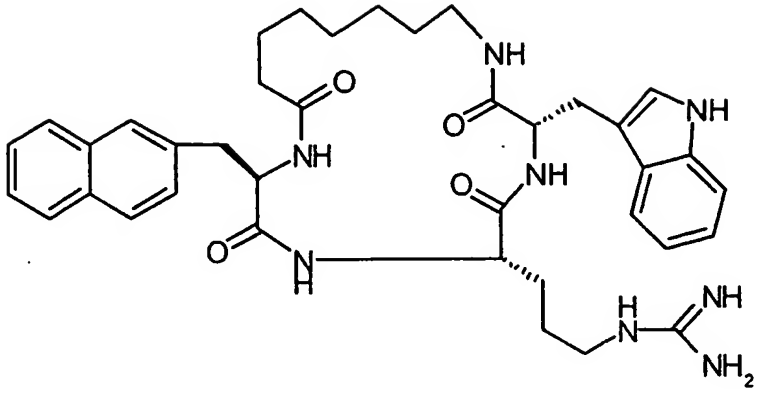

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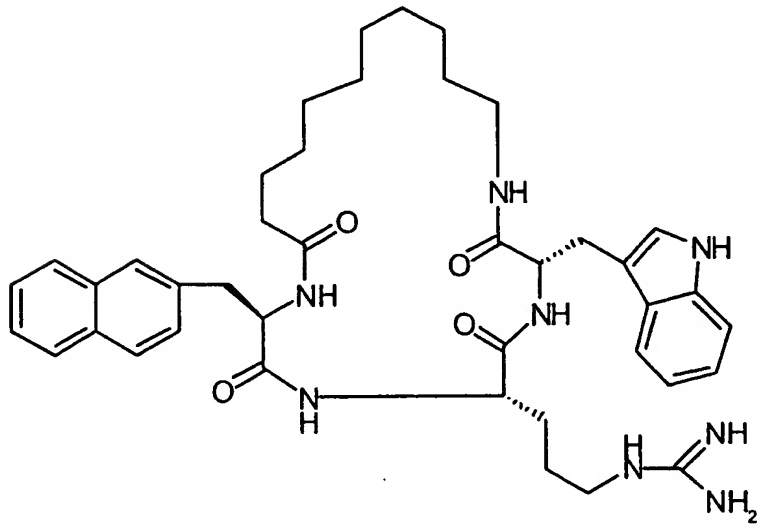

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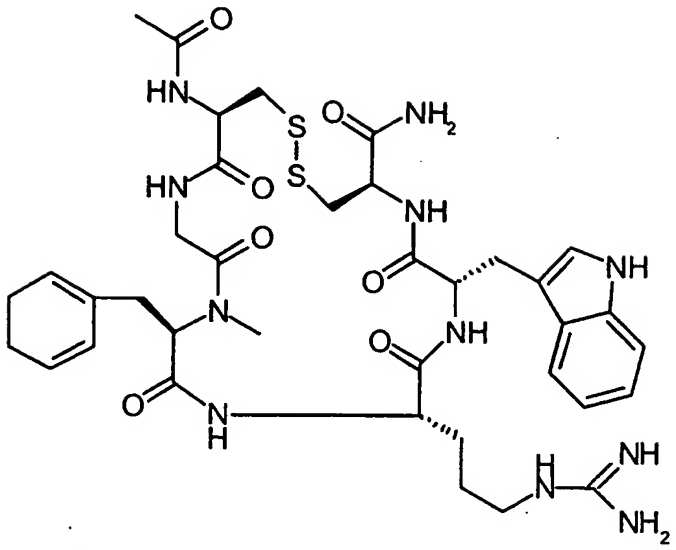

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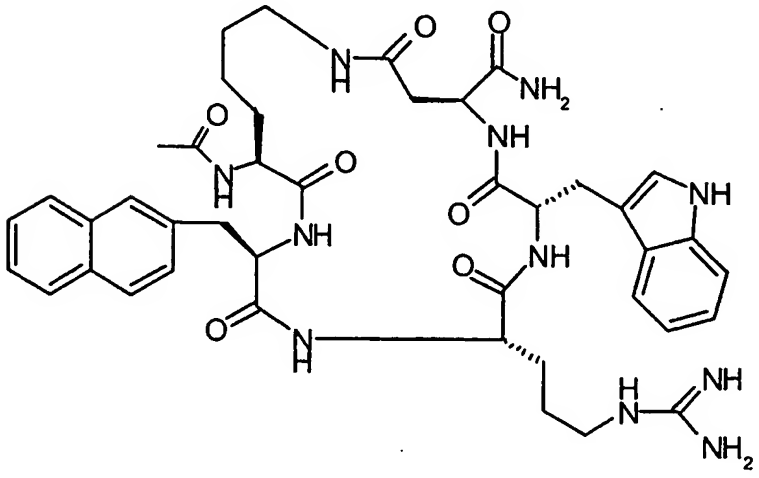

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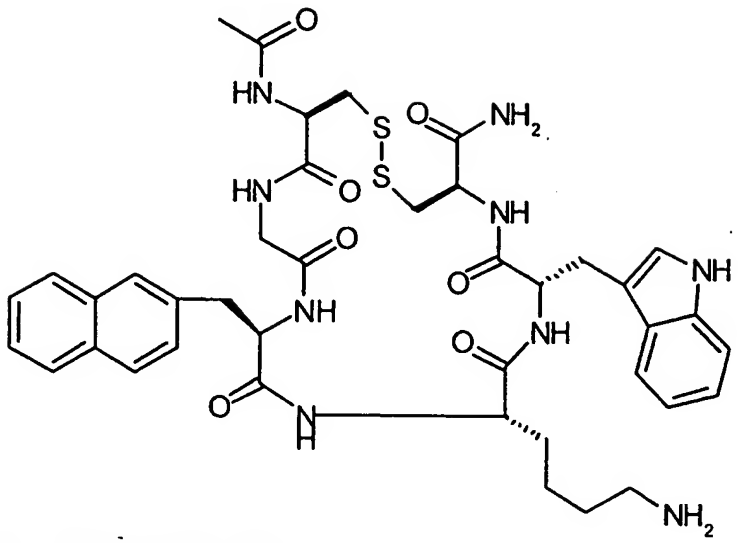

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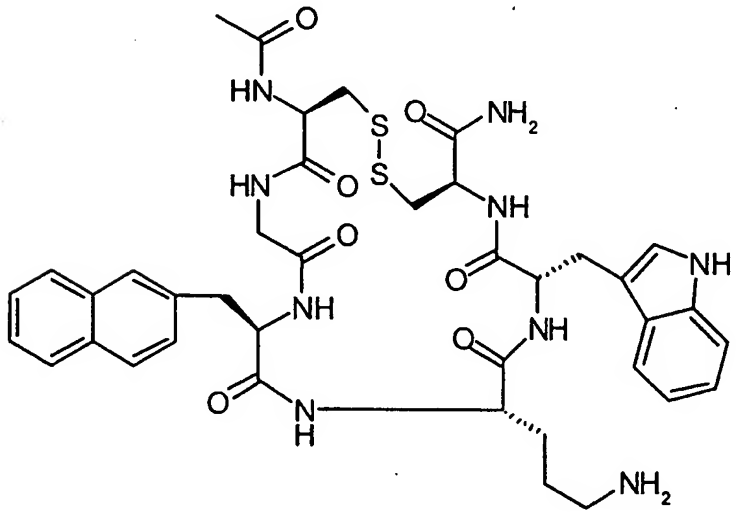

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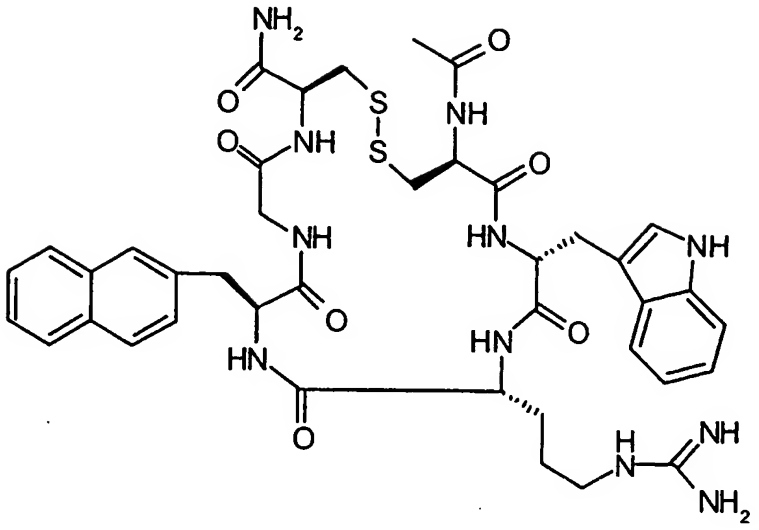
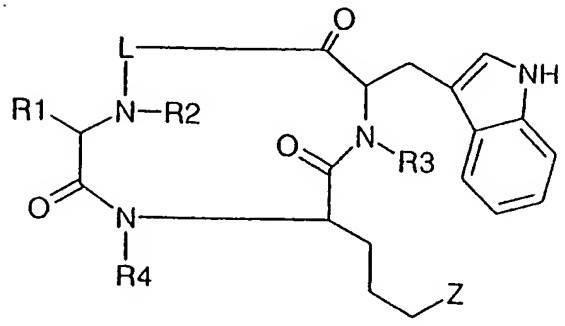
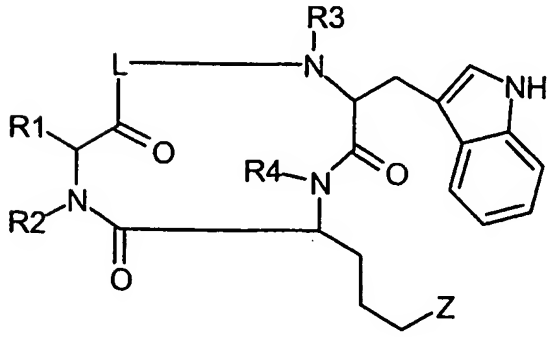


21. Use of a compound of formula (1) or formula (2)



(1)



(2)

wherein L is a linking group so as to create a cycle which contains from 18 to 21 ring-atoms;

Z is selected from $-NH_2$, $-CH_2NH_2$ and guanidino;

R1 is selected from X and $-CH_2X$ where X is H, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, heteroalkenyl, substituted heteroalkenyl, alkynyl, substituted alkynyl, heteroalkynyl, substituted heteroalkynyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, cycloalkenyl, substituted cycloalkenyl, cycloheteroalkenyl, substituted cycloheteroalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl or a functional group;

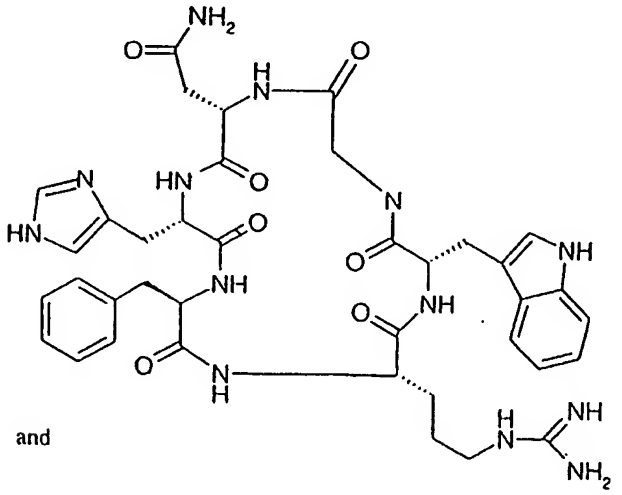
and

R2, R3 and R4 are selected from hydrogen and methyl, with hydrogen being preferred;

or a pharmaceutically acceptable salt thereof;

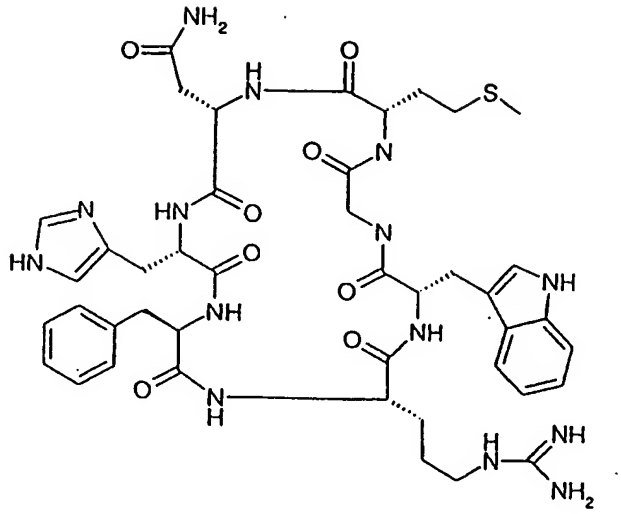
wherein

the compounds cNHdFRWG (SEQ ID NO:2) and cMNHdFRWG (SEQ ID NO:3) having structural formulae as follows



cNHdFRWG

and



cMNHdFRWG

are specifically excluded, or a compound as claimed in any one of claims 1 to 20 in the preparation of a medicament for the treatment of a weight disorder.

22. Use of a compound as defined in any one of claims 1 to 21 in the preparation of a medicament for the treatment of an eating disorder.

23. Use of a compound as defined in any one of claims 1 to 21 in the preparation of a medicament for the treatment of an addictive disorder.

24. Use of a compound as defined in any one of claims 1 to 21 in the preparation of a medicament for the treatment of inflammation.

25. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 20, together with one or more adjuvants, carriers or excipients.

26. A compound as claimed in any one of claims 1 to 20 for use in a method of treatment of the human or animal body or a diagnostic method practised on the human or animal body.

27. A method of treating a weight disorder comprising administering an effective amount of a compound as defined in any one of claims 1 to 21.

28. A method of treating an eating disorder comprising administering an effective amount of a compound as defined in any one of claims 1 to 21.

29. A method of treating an addictive disorder comprising administering an effective amount of a compound as defined in any one of claims 1 to 21.

30. A method of treating inflammation comprising administering an effective amount of a compound as defined in any one of claims 1 to 21.

Figure 1

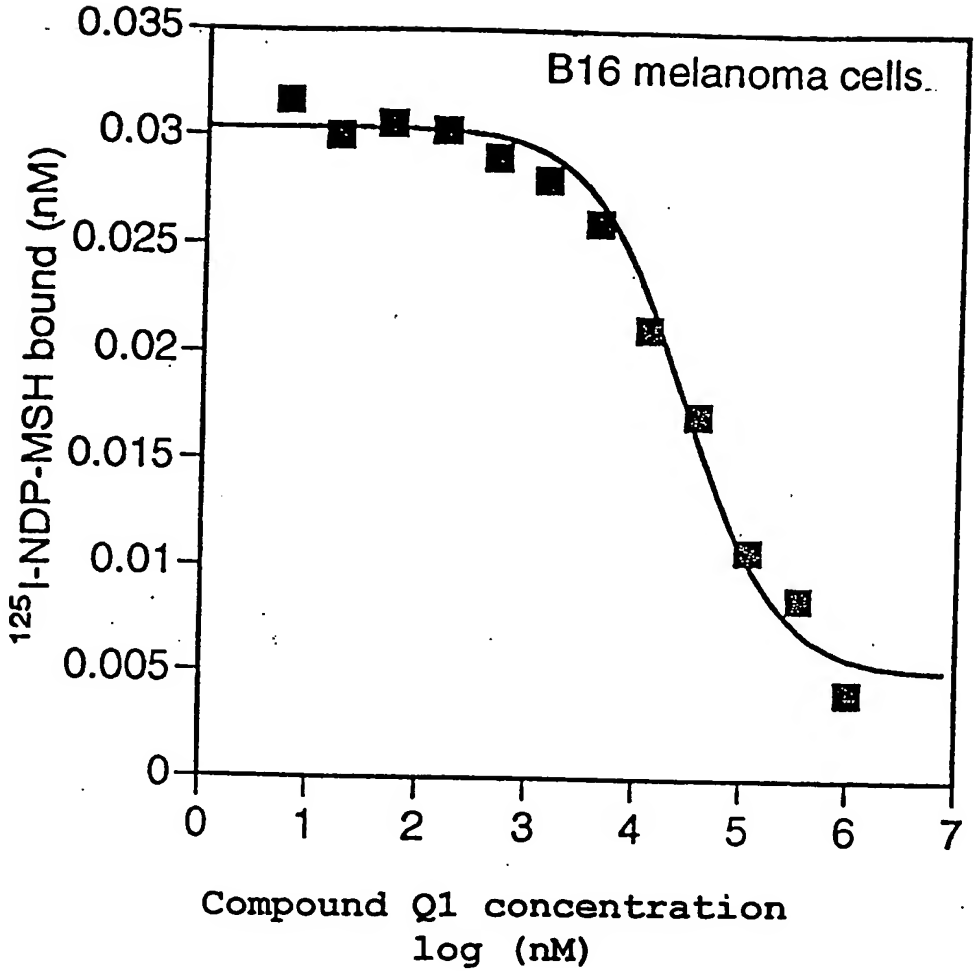

B16 melanoma cells
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
^{125}I-NDP-MSH bound (nM)
0
1
2
3
4
5
6
7
Compound Q1 concentration
log (nM)

Figure 2

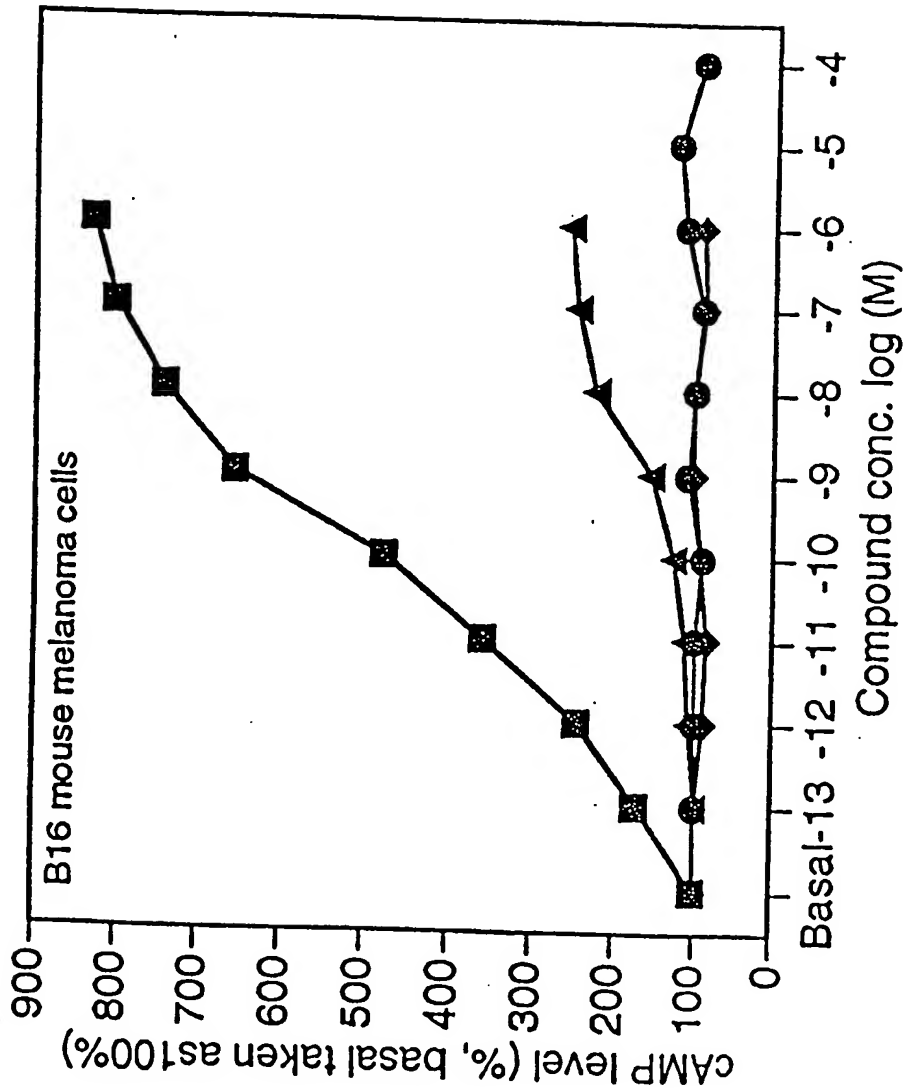

αMSH
Q1
αMSH + Q1 0.01μM
αMSH + Q1 0.1μM
B16 mouse melanoma cells
cAMP level (%, basal taken as100%)
900
800
700
600
500
400
300
200
100
0
Basal-13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Compound conc. log (M)

Figure 3

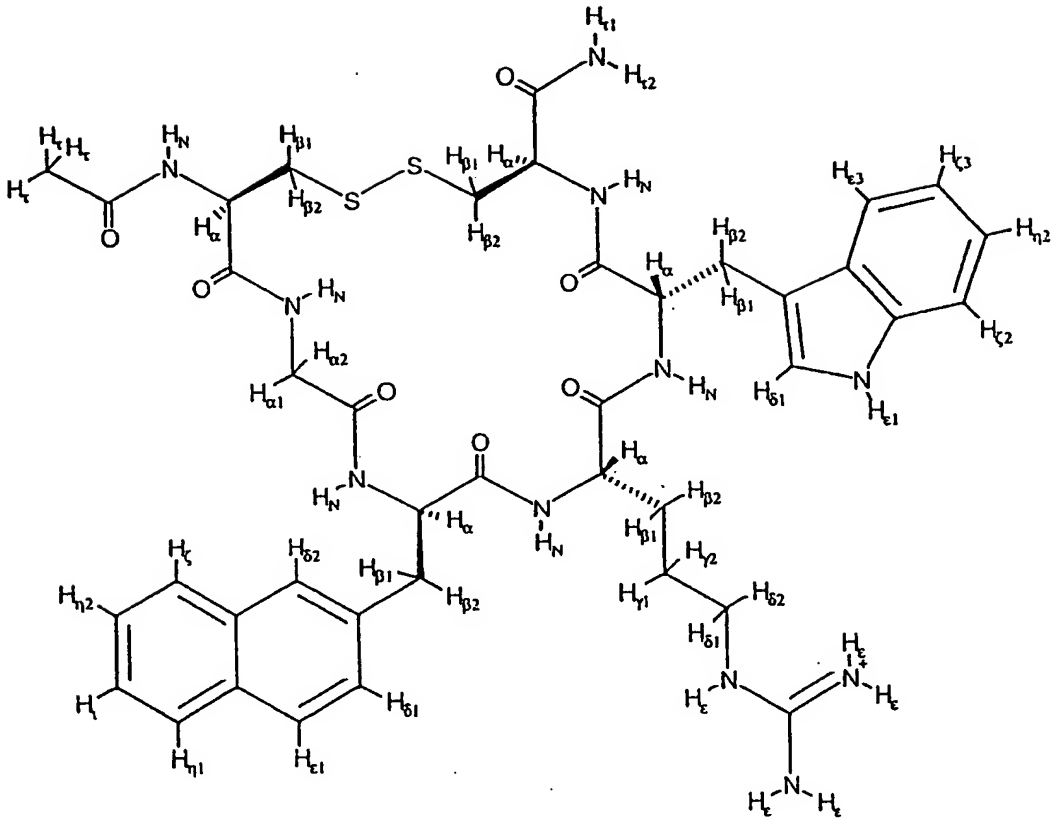


Figure 4

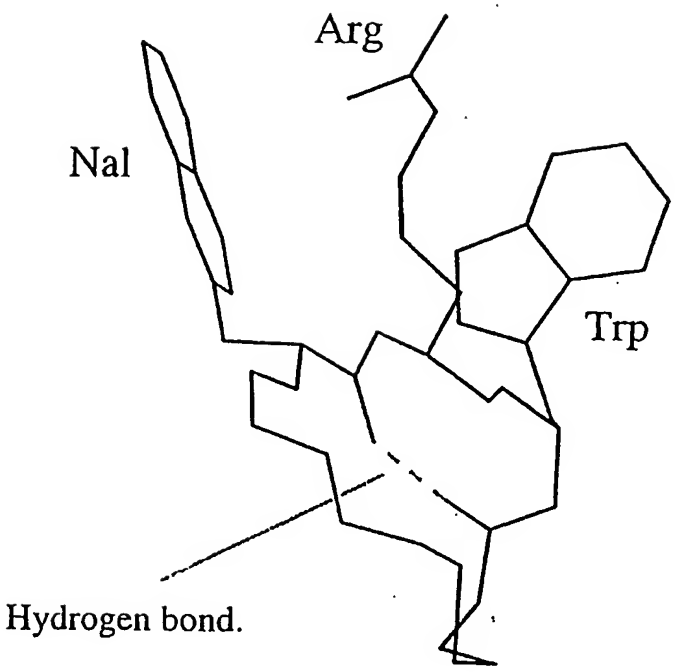


Figure 5

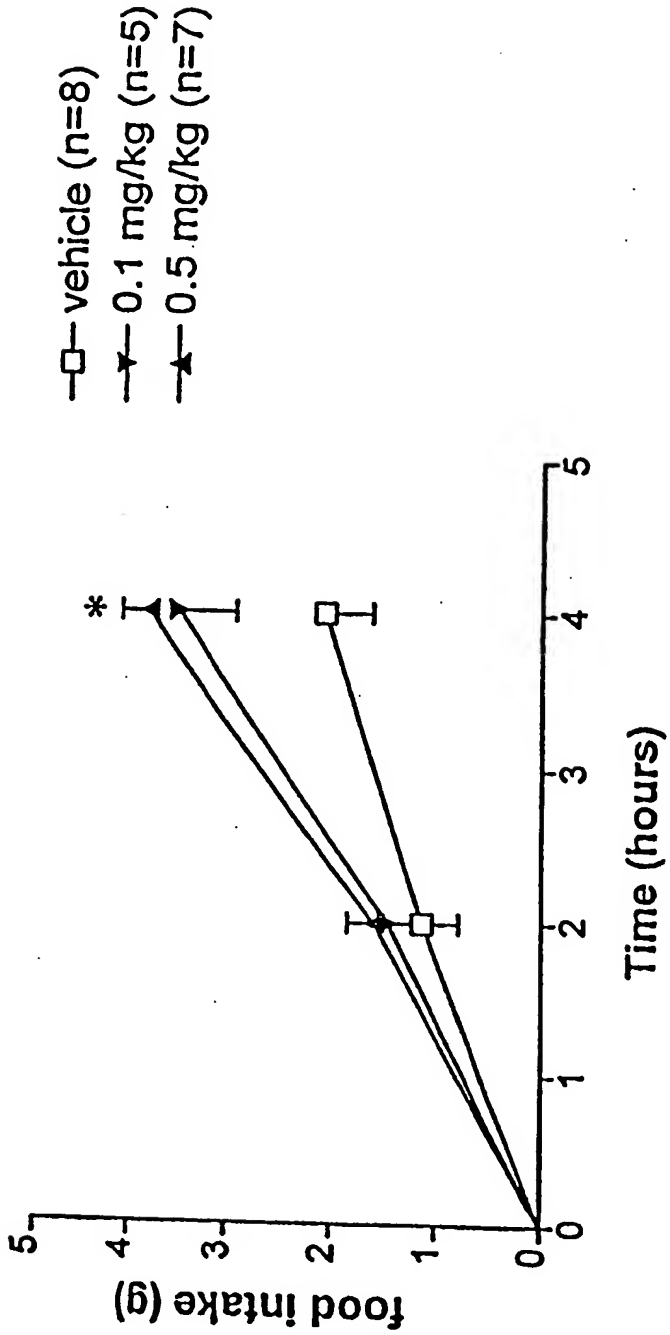
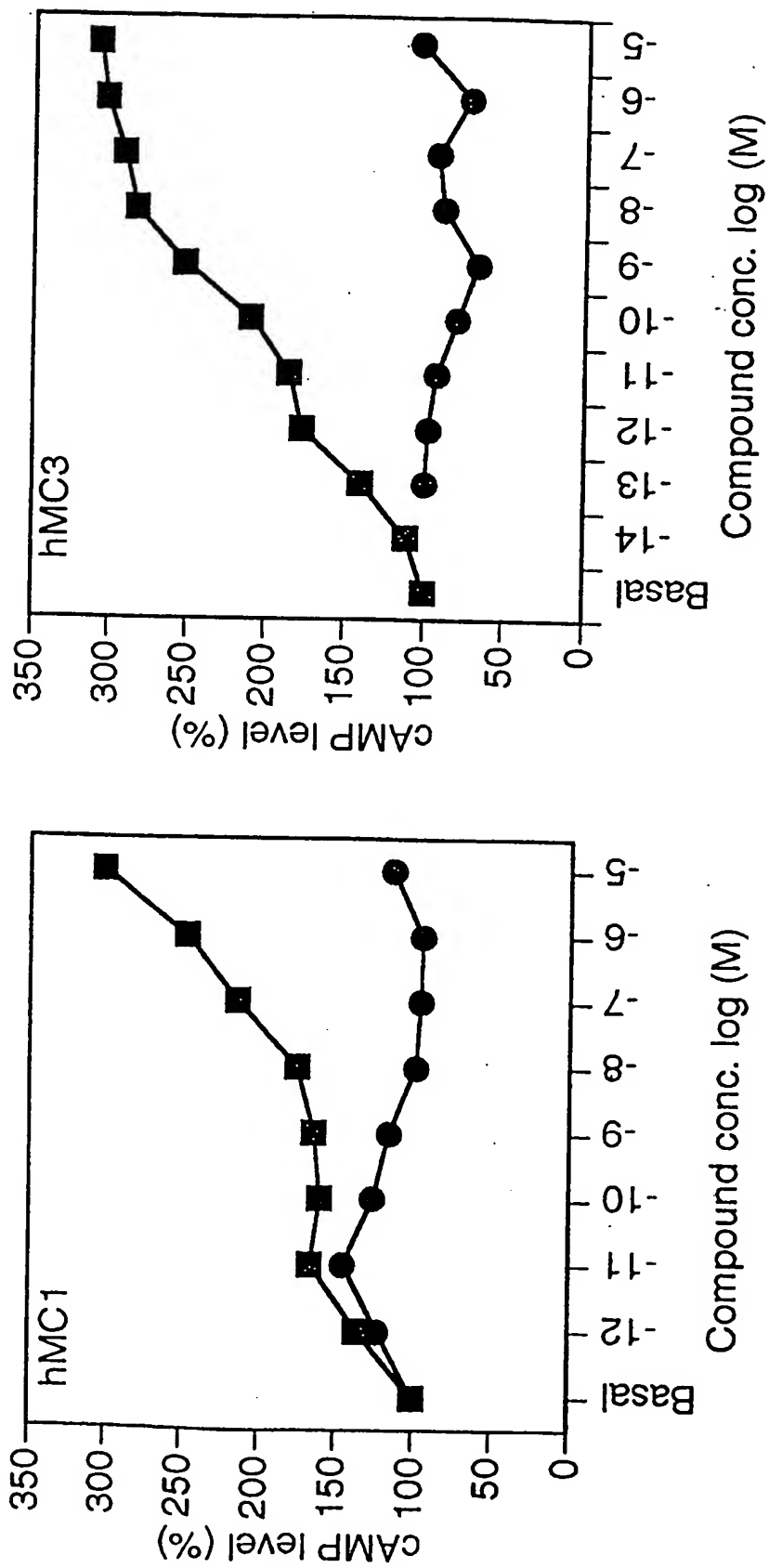

vehicle (n=8)
0.1 mg/kg (n=5)
0.5 mg/kg (n=7)
*
food intake (g)
Time (hours)
0
1
2
3
4
5

Figure 6


hMC3
cAMP level (%)
350
300
250
200
150
100
50
0
Basal
-14
-13
-12
-11
-10
-9
-8
-7
-6
-5
Compound conc. log (M)
hMC1
cAMP level (%)
350
300
250
200
150
100
50
0
Basal
-12
-11
-10
-9
-8
-7
-6
-5
Compound conc. log (M)

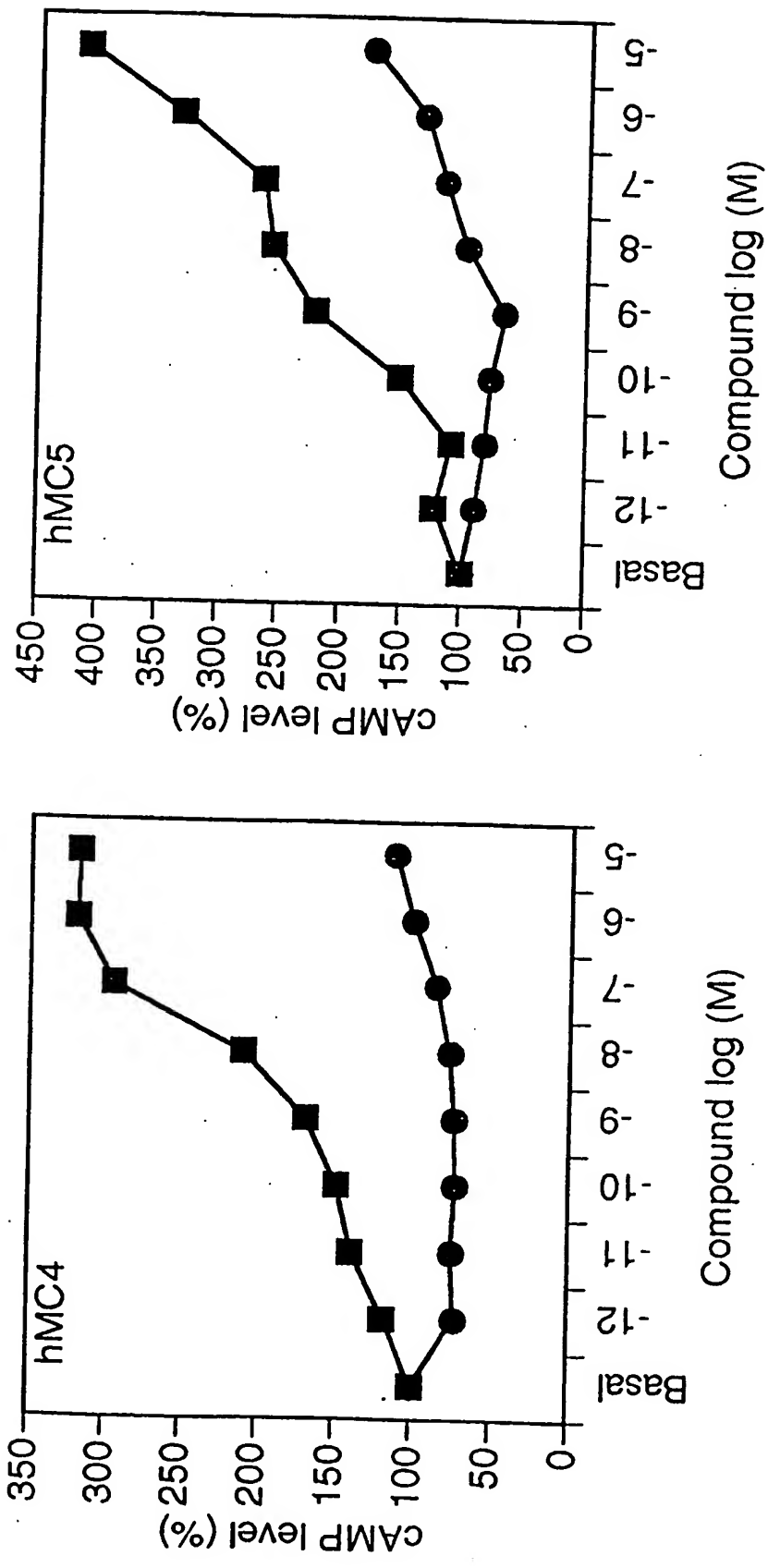

hMC4
cAMP level (%)
0
50
100
150
200
250
300
350
Basal
-12
-11
-10
-9
-8
-7
-6
-5
Compound log (M)
hMC5
cAMP level (%)
0
50
100
150
200
250
300
350
400
450
Basal
-12
-11
-10
-9
-8
-7
-6
-5
Compound log (M)

Figure 7

SEQUENCE LISTING

<110> Melacure Therapeutics AB

<120> Compounds for control of eating, growth and body weight

<130> Listing for PCT application as filed

<140>
<141>

<150> UK 9827500.1
<151> 1998-12-14

<160> 24

<170> PatentIn Ver. 2.1

<210> 1
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<212> PRT
<213> Frog

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<220>
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<222> (4)
<223> D-Phe

<220>
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<222> (5)
<223> Nle

<220>
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<222> (7)
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<400> 1
Xaa Asp Trp Phe Xaa Trp Lys

1 5

<210> 2
<211> 6
<212> PRT
<213> Artificial Sequence

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<220>
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<223> Peptide bond between Asn and Gly forming a cyclic peptide

<400> 2
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1 5

<210> 3
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<220>
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<220>
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<222> (1)..(7)
<223> Peptide bond between Met and Gly forming a cyclic peptide

<400> 3
Met Asn His Phe Arg Trp Gly
1 5

<210> 4
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<220>
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1

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<220>
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<220>
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Cys Gly Ala Arg Trp Cys

1 5

<210> 6
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<220>
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<223> D-Phe

<220>
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Cys Gly Phe Arg Trp Cys
1 5

<210> 7
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<220>
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<222> (3)
<223> 3,4-dichloro-Phe

<220>
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Cys Gly Phe Arg Trp Cys
1 5

<210> 8

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<212> PRT
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<220>
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<220>
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1 5

<210> 9
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receptors

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1 5

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<400> 10
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1 5

<210> 11
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<220>
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<223> L-penicillamine

<220>
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<222> (6)
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<400> 11
Cys Gly Ala Arg Trp Xaa
1 5

<210> 12
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<220>
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<223> L-penicillamine

<220>
<223> Description of Artificial Sequence:Compound having affinity for melanocyte stimulating hormone receptors

<220>
<221> MOD_RES
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<400> 12
Xaa Gly Ala Arg Trp Xaa
1 5

<210> 13
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1 5

<210> 14
<211> 6
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1 5

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<223> D-3-(1-naphthyl)alanine

<220>
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<222> (6)
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1 5

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<222> (1)..(6)

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1 5

<210> 17
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<223> D-3-(2-naphthyl)alanine

<220>
<221> MOD_RES
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<223> AMIDATION

<220>
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<222> (1)..(6)

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<400> 17
Cys His Ala Arg Trp Cys
1 5

<210> 18
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<222> (1)
<223> 8-amino-octanoic acid

<220>
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<222> (2)
<223> D-3-(2-naphthyl)alanine

<220>
<221> MOD_RES
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<223> Ring formed through peptide bond between 8-amino-octanoic acid and Trp

<220>
<223> Description of Artificial Sequence:Compound having affinity for melanocyte stimulating hormone receptors

<400> 18
Xaa Ala Arg Trp
1

<210> 19
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<213> Artificial Sequence

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<220>

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<220>
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<223> Ring formed through peptide bond between
11-amino-undecanoic acid and Trp

<220>
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affinity for melanocyte stimulating hormone
receptors

<400> 19
Xaa Ala Arg Trp

1

<210> 20
<211> 6
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affinity for melanocyte stimulating hormone
receptors

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1 5

<210> 21
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<220>
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<220>
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<220>
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<220>
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1 5

<210> 22
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